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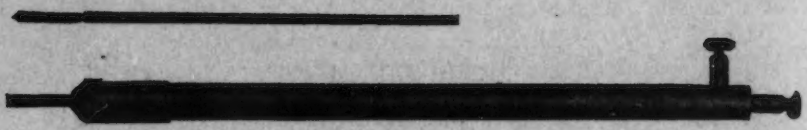

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AMINO-ACIDS IN NUTRITION

V. Nutritive Value of Edestin (globulin from hemp seed):[1] Cystine and Lysine as Growth-Limiting Factors in that Protein

BARNETT SURE

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville

Received for publication February 27, 1921

For his plan to study the rôle of proline in nutrition the author originally chose three proteins, namely, arachin, lactalbumin and edestin. Arachin and lactalbumin were fortified with cystine to prevent that amino-acid from being a growth-limiting factor, so that a response to the pyrrolidine nucleus might be made possible. Surprisingly, normal growth was obtained with lactalbumin on 18 and 12 per cent levels. On reducing the plane of intake to 9 per cent, however, a response to cystine was procured only in the presence of tyrosine (1). The results of the work on the nutritive value of lactalbumin invited similar experimental studies in connection with the possible cause of the nutritive failure of edestin on strictly synthetic diets.

The rations employed in this investigation are as strictly synthetic as it was possible to make them. The proteins used served as the only source of nitrogen with the exception of the small amounts introduced by the alcoholic extracts of the wheat embryo to furnish water-soluble

[1] The edestin used in rations 32 and 82 was prepared by extracting with 10 per cent NaCl, precipitated with 5 volumes of water, freed of salt, and then purified with 50 per cent alcohol. The edestin used in all of the other rations was further purified by reëxtracting with 20 per cent NaCl.

vitamin B. The amounts of foreign nitrogen thereby introduced constituted only 2 per cent of the total protein. But even when fortified with this proportion of nitrogen of foreign source, edestin was found inadequate for growth on as high levels of protein intake as 12 and 18 per cent of the total ration.

Although Osborne and Mendel procured a certain amount of success with edestin employing natural "protein-free-milk," they report an improvement in the character of growth on the addition of lysine to their edestin-protein-free milk rations (2). Consequently, lysine as well as cystine and tyrosine were used in this investigation. The results of the experiments are given in the following charts:

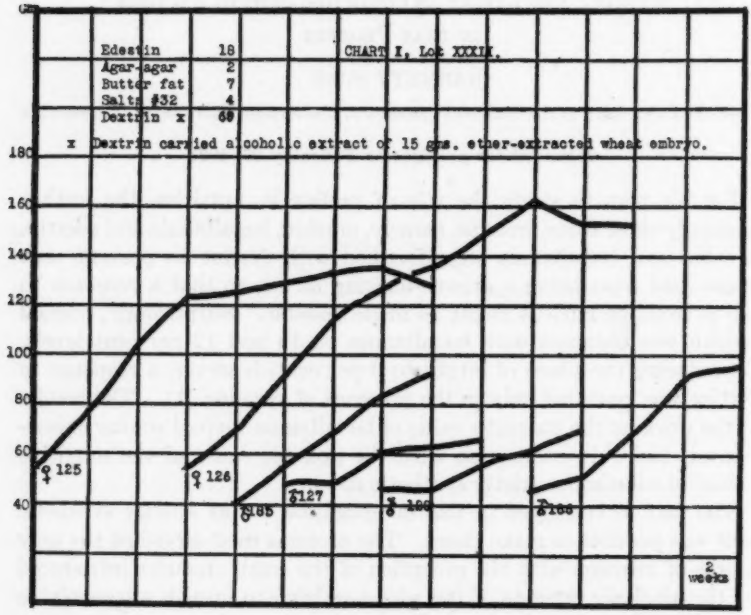

Chart I, Lot 32. This chart indicates that on as high a plane of protein intake as 18 per cent edestin is very inadequate for good growth. Rats 125 and 126 have made a fair amount of growth during the first 6 to 8 weeks, but show subsequent failure.

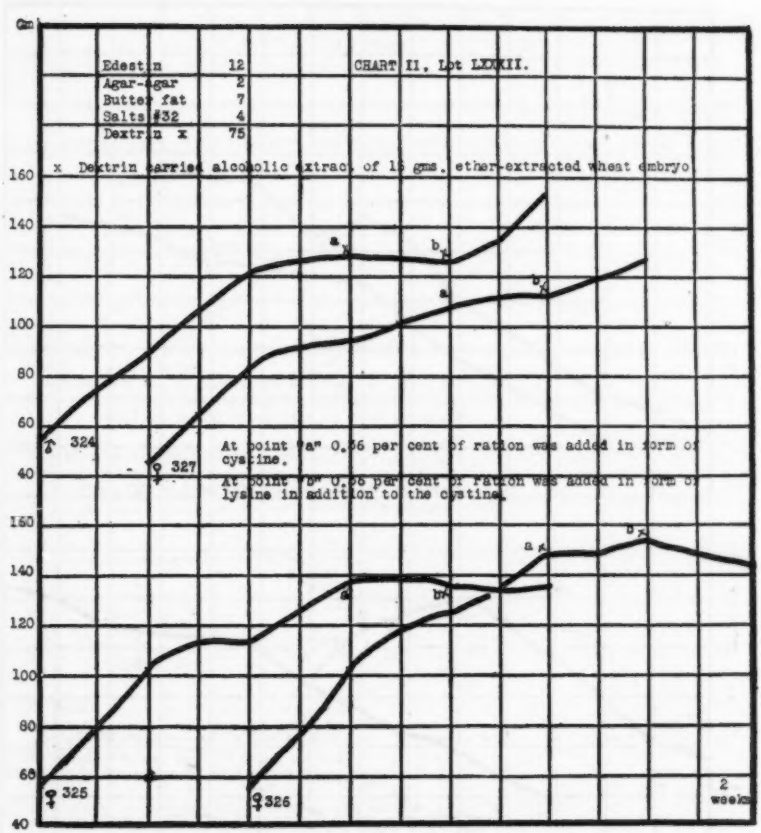

Chart II, Lot 82. Even on reducing the level of edestin to 12 per cent some animals show a fair amount of growth during the first 4 to 6 weeks, but later, without exception, reach practically maintenance curves. At point *a*, 0.4 per cent of the ration was added in the form of cystine, but there was no response. At point *b*, 0.36 per cent of the ration was introduced in the form of lysine (as the free acid) in addition to the cystine, and it will be noted, animal 324 shows a marked response, animal 327 a slow but definite response, while rats 325 and 326 did not respond at all.

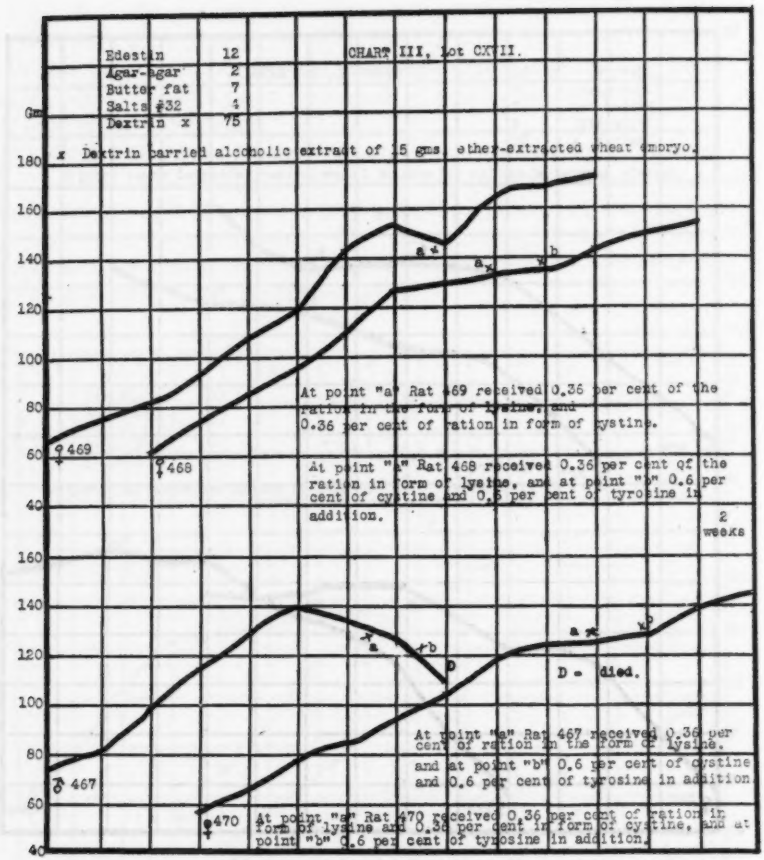
Edestin 12
Agar-agar 2
Butter fat 7
Salts #32 4
Dextrin x 75
CHART III, Lot CXVII.
x Dextrin parried alcoholic extract of 15 gms ether-extracted wheat embryo.
Gm
180
160
140
120
100
80
60
40
♀469
♀468
♂467
♀470
At point "a" Rat 469 received 0.36 per cent of the ration in the form of lysine, and 0.36 per cent of ration in form of cystine.
At point "a" Rat 468 received 0.36 per cent of the ration in form of lysine, and at point "b" 0.6 per cent of cystine and 0.6 per cent of tyrosine in addition.
2 weeks
D = died.
At point "a" Rat 467 received 0.36 per cent of ration in the form of lysine.
and at point "b" 0.6 per cent of cystine and 0.6 per cent of tyrosine in addition.
At point "a" Rat 470 received 0.36 per cent of ration in form of lysine and 0.36 per cent in form of cystine, and at point "b" 0.6 per cent of tyrosine in addition.

Chart III, Lot 117. Since two animals did not respond to a cystine-lysine addition, it was decided to repeat the above experiment and employ tyrosine in addition, if necessary. Rat 469 shows an increase from point *a* after a lysine-cystine addition. Rat 468 received at point *a* an addition of lysine alone without showing any signs of increase of body weight 2 weeks after the addition. Since, in the experience of the writer, two weeks was found to be ample time for securing amino-acid responses, and in order to prevent animal 468 from falling into a bad nutritive state, on account of a possible deficiency of a number of other amino-acids, cystine and tyrosine were introduced in the ration in addition to the lysine, and a slow but definite response was obtained in the case of both animals. Rat 467 evidently was already in a malnutritive condition when the amino-acid

additions were made, since it died 2 weeks after the lysine-cystine-tyrosine addition. It is not at all clear from this experiment whether a response to cystine-lysine additions might not have been obtained in the absence of tyrosine. This fact is brought out more clearly in charts VI, VII and VIII.

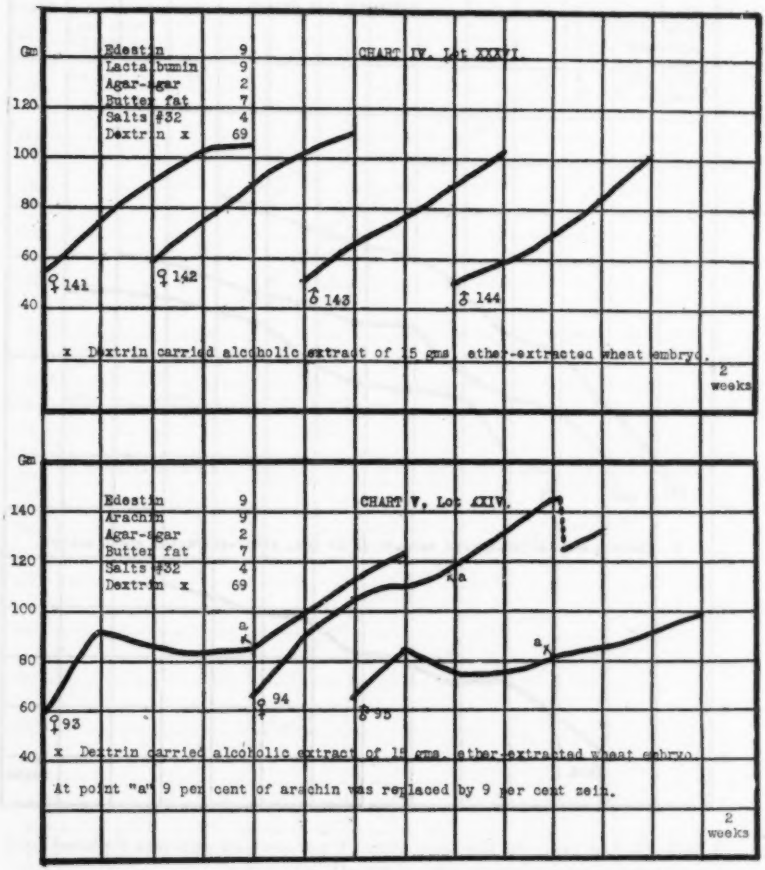

Chart IV, Lot 36. This chart indicates that lactalbumin shows very little supplementary value to edestin (see chart I, lot 32), in the absence of amino-acids.

Chart V, Lot 24. Arachin, like lactalbumin, does not furnish amino-acids to improve the nutritive character of edestin. At point *a*, 9 per cent of arachin was replaced by an equivalent amount of zein, and no marked change in the nature of growth is demonstrable.

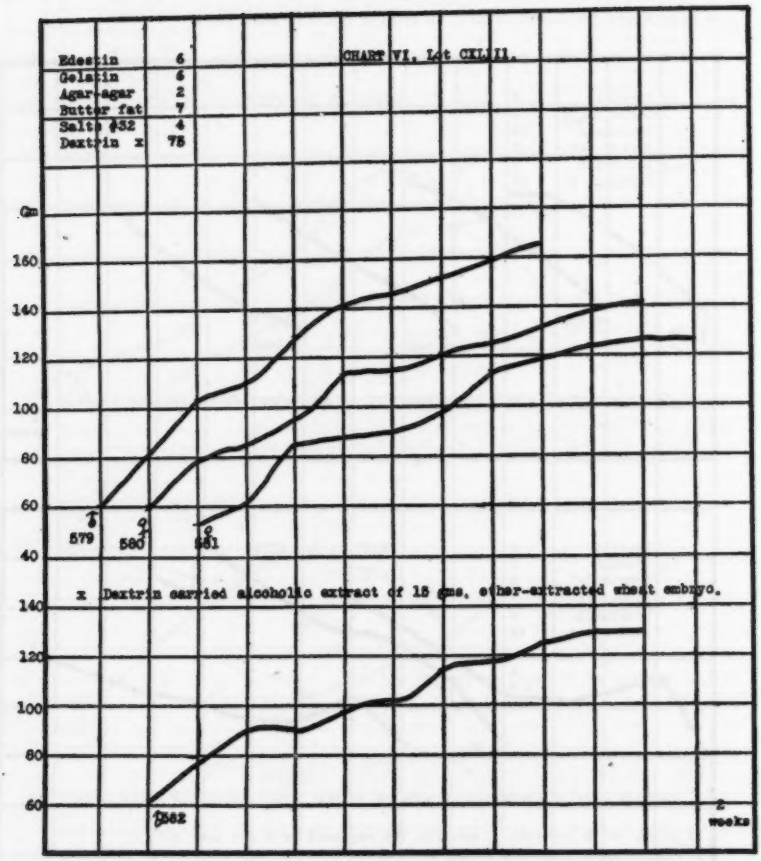

Chart VI, Lot 143. In this experiment 6 per cent edestin was replaced by 6 per cent gelatin, and a fair amount of growth is apparent.

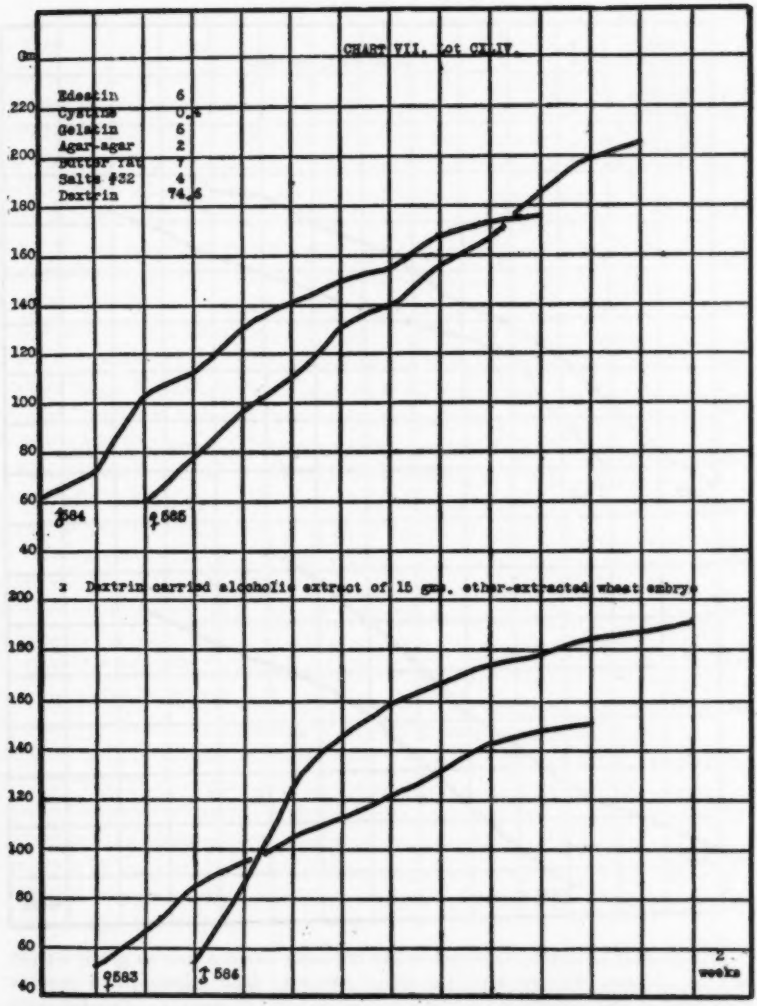

Chart VII, Lot 144. This ration is a duplicate of the one just preceding, lot 143, with the exception that 0.4 per cent cystine replaced an equivalent amount of dextrin. The difference in growth between this lot and lot 143 is very striking indeed. Evidently gelatin is furnishing a number of amino-acids deficient in edestin, which must be supplied, so the beneficial effect from a cystine addition might become more apparent.

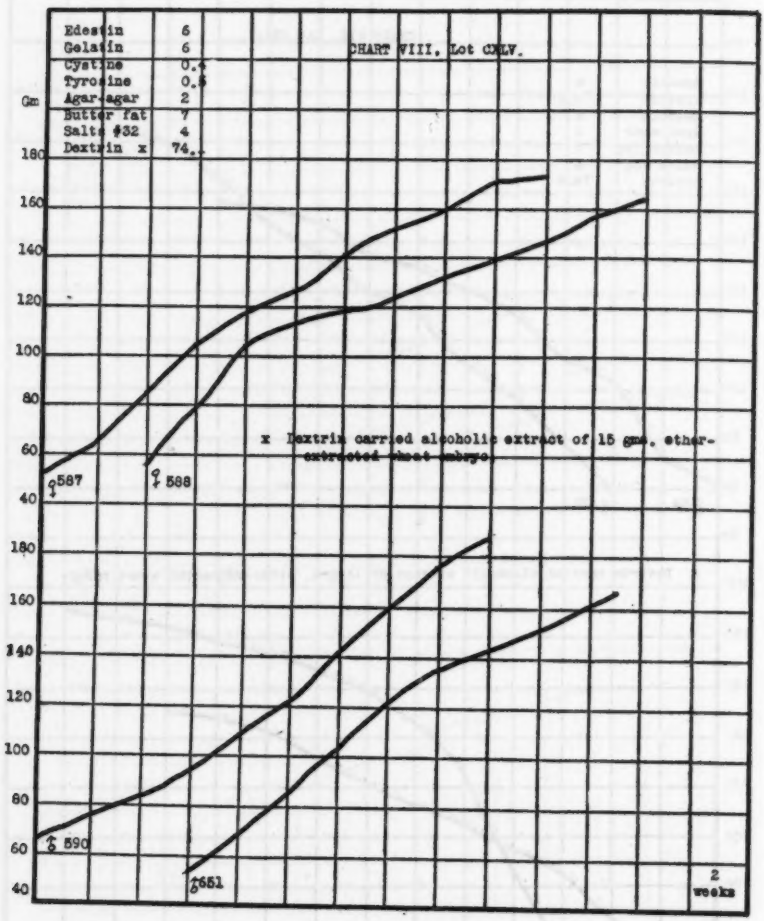

Chart VIII, Lot 145. This ration has the same composition as given lot 144 and in addition 0.5 per cent tyrosine. Accurate food consumption records were kept of this experiment and the preceding lots 143 and 144. This lot (145) consumed practically the same amount of food as lot 143, but less than lot 144, and allowing for difference in food consumption, this lot made as good growth, if not a little better than lot 144. No pronounced beneficial effect, however, from the addition of tyrosine could be detected.

Discussion. Preliminary work on the nutritive value of edestin showed that this protein is of poor quality. The addition of cystine brought about no response, but the introduction of lysine in ration 82, in the presence of cystine, was accompanied by an increase in growth in the case of two animals. Two animals did not respond at all. This experiment was repeated for the purpose of incorporating tyrosine in the ration. Since Osborne and Mendel have failed with lactalbumin as well as edestin on strictly synthetic diets (3), and since tyrosine and cystine have been found to be the determining growth-limiting factors in lactalbumin (1), a further attempt was made to determine whether a response to cystine-lysine additions could be unfailingly procured in the presence of tyrosine. From his experience in his studies of amino-acids in nutrition, the author has observed that individuals vary in the nature of their response to isolated or multiple additions of amino-acids used to fortify pure proteins. Occasionally an animal will make normal growth, while 10 to 12 individuals in the same experiment will fail solely on account of a deficiency of cystine. The inevitable conclusion to be drawn, then, is that individuals vary in their power of conserving certain nuclei of the protein molecule. Although it has been clearly demonstrated that lactalbumin is deficient in the cystine complex, one animal on an 18 per cent level made normal growth in the absence of cystine (1). This animal must have had the capacity of conserving the cystine out of the very small amount contained in the milk albumin. The reason two animals failed to respond to a cystine-lysine addition was attributed to the fact that they lacked the power to conserve the hydroxy-phenyl nucleus, in which edestin is not very abundant, to the same extent as the animals which responded in the absence of tyrosine. The mechanism by which certain individuals display a relatively greater efficiency than others in conserving the links of the protein chain used for the construction of body tissue is difficult to conceive, unless it be related to the nature of enzyme action, which in turn may be influenced by the hormones of the internal secretions.

In order to eliminate the possibility of certain individuals suffering from a tyrosine deficiency, lot 117 was subjected to this inquiry, and by inspection of chart III it will be noted that animal 468 shows no change in the character of growth after the addition of isolated lysine at point *a*, and animal 470 shows no response at point *a* after a cystine-lysine addition. Definite response, however, is apparent after point *b* to a cystine-lysine-tyrosine addition. Animal 469 responded in the absence of tyrosine. Rat 467 died soon after the cystine-lysine-tyrosine addi-

tion, probably because of the fact that its body tissue was already injured to the extent that recovery after the introduction of the amino-acid combination was not possible. It is not apparent, however, that the response secured after point *b* in the case of rat 468 might not have been possible in the absence of tyrosine. Whether or not tyrosine is deficient in edestin will be more evident from the following series of experiments.

In a previous communication in connection with studies on amino-acid deficiencies in the proteins of the Georgia velvet bean (4) it has been demonstrated that, while no success was obtained to straight cystine additions made at the optimum plane of seed intake, a slow but definite response to cystine addition was secured in the presence of gliadin and zein, and a remarkable response which was continuous was obtained in the presence of gelatin. Since gelatin is quite satisfactory for its lysine content (5), and since in this study of the nutritive value of edestin responses to cystine were obtained only in the presence of lysine, gelatin was incorporated in the diet as a possible source of other amino-acids, including lysine, so that it might be more apparent whether cystine or tyrosine or both of these amino-acids are growth-limiting factors in the protein under investigation. Three experiments were carried out. In one (lot 143), without amino-acid additions, 6 per cent edestin was replaced by 6 per cent gelatin. A lower level of protein intake was employed in these experiments, with a view of producing greater demand for cystine or tyrosine or both. Another ration, lot 144, contained 6 per cent edestin and 6 per cent gelatin and in addition 0.4 per cent cystine. Lot 145 received the same ration as lot 144 and in addition 0.5 per cent tyrosine.

For these experiments a type of feeding pan[2] was employed which allows very little, if any, waste of food. When all bedding is removed, scarcely any part of the ration can be detected scattered in the cage. Just enough food was allowed the animals, so that they could have a liberal supply for consumption and still have very little left in their pans on the following day. With this method of careful feeding, the experimental error in keeping record of food consumption could scarcely be over 3 to 5 per cent. The results are shown in the following tables:

[2] The type of pan employed was recently devised and is being successfully used by the Department of Agricultural Chemistry of the University of Wisconsin in their nutrition studies in experiments where the matter of food consumption is an important factor.

TABLE 1

Showing gains in weight in grams

M = male; F = female

PROTEIN PER CENT	LOT NUMBER	RAT NUMBER	SEX	WEIGHT OF ANIMALS					
				Initial	Four weeks	Eight weeks	Twelve weeks	Sixteen weeks	Twenty weeks
Edestin, 6.0	143	582	M	61	89	104	115	127	131
Gelatin, 6.0		581	F	53	86	91	114	124	125
		579	M	60	103	123	140	153	167
		580	F	59	84	114	121	132	141
Edestin, 6.0	144	584	M	62	101	132	150	169	172
Cystine, 0.4		585	F	60	96	128	156	186	206
Gelatin, 6.0		583	F	51	86	106	120	141	148
		586	M	53	124	160	173	183	186
Edestin, 6.0	145	587	F	53	87	117	140	159	170
Cystine, 0.4		588	F	57	106	118	132	146	161
Tyrosine, 0.5		590	M	64	75	120	146	181	
Gelatin, 6.0		651	M	52	75	125	141	161	

TABLE 2

Showing gain in grams per gram of protein intake

PROTEIN PER CENT	LOT NUMBER	GAIN IN WEIGHT	AMOUNT OF PROTEIN CONSUMED	GAIN PER GRAM OF PROTEIN INTAKE
		grams	*grams*	*grams*
Edestin, 6.0 Gelatin, 6.0	143	331	553.2	0.598
Edestin, 6.0 Cystine, 0.4 Gelatin, 6.0	144	486	623.4	0.783
Edestin, 6.0 Cystine, 0.4 Tyrosine, 0.5 Gelatin, 6.0	145	451	565.2	0.798

Charts VI, VII and VIII clearly indicate the remarkable difference in growth due to the presence of cystine in the rations when administered in the presence of gelatin. From the nature of the curves of charts VII and VIII it would appear that the animals that received the cystine and tyrosine additions did not do quite as well as the lot that received cystine in the absence of tyrosine. An examination of table 2, however, shows that this is not the case. When the amount of protein consumed by these two groups of animals is taken into consideration, and the gain in weight calculated per gram of protein intake, it is evident that the tyrosine-fed rats did even a little better than the animals that received the cystine addition alone. The difference, however, is so slight that it may well fall within the experimental error of the method employed of keeping record of food consumption, and the conclusion must be drawn, therefore, that no pronounced additional advantage was derived by the introduction of tyrosine in the edestin-gelatin combination. The animals that received the cystine addition (lot 144) made 31.1 per cent more growth, and those that received the cystine-tyrosine addition (lot 145), 33.4 per cent more growth than the control (lot 143). Cystine, then, is unquestionably a growth-limiting factor in edestin, a fact which becomes absolutely apparent only when administered in the presence of a protein with a high lysine content, such as gelatin. Gelatin presumably is also furnishing edestin amino-acids other than lysine, which are also deficient in the above-mentioned protein, because, while some responses are obtained at times to straight cystine-lysine additions, it is not always possible to secure them, since there is probably a demand for other protein groupings.

It may still be argued that the responses obtained to amino-acid additions in the presence of gelatin should be credited to gelatin rather than to edestin; in other words, that cystine improved the deficient character of gelatin rather than the poor quality of edestin. This difficulty was circumvented by the following experiment. Gelatin was fed at 12 per cent planes of intake, and even in the presence of 0.4 per cent cystine, 0.4 per cent tryptophane and 0.6 per cent tyrosine, only maintenance was secured for several weeks, after which period all the animals began to fail very badly. The beneficial results procured by the introduction of cystine in the edestin-gelatin rations must be credited to edestin, then, rather than to gelatin.

Neither lactalbumin, arachin nor zein, in the absence of amino-acids, improved the deficient quality of edestin.

SUMMARY

1. Edestin when fed as 12 and 18 per cent of a ration, carrying 2 per cent of the total protein in the form of nitrogen of unknown source in alcoholic extract of wheat embryo to furnish water-soluble vitamin B, was found to be inadequate for growth.

2. Responses to cystine were obtained, but only in the presence of lysine.

3. When cystine was administered in the edestin rations in the presence of gelatin, having a high lysine content, the growth was increased 31.4 per cent.

4. *Cystine and lysine at least are two amino-acids responsible for the deficient quality of edestin.*

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XIII. The Innervation of the Cardia and the Lower End of the Esophagus in Mammals

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While investigating the reflex control of the cardia and the lower esophagus in mammals, it soon developed that some of the results obtained seemed to require both motor and inhibitory innervation of the cardia-esophagus from other sources than the vagi nerves. On reviewing the literature on the sympathetic innervation of the cardia and lower esophagus the reports were found so contradictory that it was deemed necessary to reinvestigate the subject in the available laboratory mammals (dog, cat, rabbit), before further advance in the physiology and pathology of the nervous control of this region of the gut could be made.

The literature. The literature on the relation of the vagi efferents to the cardia and the lower esophagus in mammals is fairly consistent. The vagi supply both motor and inhibitory efferents to the cardia, and probably to that part of the lower esophagus which is made up of a system of unstriped musculature and local ganglia (plexus of Auerbach). According to some investigators this vagus system is the sole efferent nerve supply to the cardia and esophagus; according to others, sympathetic efferents are also present.

In his monograph on the *Involuntary Nervous System* (19) Gaskell says, (p. 51): "As far as concerns the esophagus and its termination in the cardiac sphincter of the stomach, there is no evidence that sympathetic motor fibres reach to this region. The motor supply is entirely from motor cells connected with the vagus nerve." Gaskell also states (p. 74) that Cannon has shown that "the cardiac end of the stomach inclusive of the cardiac sphincter, is innervated both by motor and inhibitory fibres in connection with the vagus nerve alone." I can

find no such report or conclusion in Cannon's work on the gut, unless the following statement may be so construed: "cutting off all vagus supply, except one recurrent laryngeal, destroys the extrinsic innervation of the gullet between the base of the neck and the cardia" (9, p. 29). In fact, Cannon does not consider the sympathetic system in his work on the nervous control of the esophagus and cardia. The only specific reference to it by Cannon appears to be the following: "Langley has reported that the cardiac sphincter is relaxed when adrenalin is given; and as the effect of adrenalin is an indicator of the presence and function of sympathetic nerve fibres, the conclusion is justified that the cardia has a sympathetic supply which causes a relaxation." (9, p. 34).

Page-May (40), in 1904, on the basis of extensive work on monkeys, dogs, cats and rabbits, using curare and various anesthetics, graphic registration and direct observation, came to the conclusion that the splanchnic efferents had no action either on the cardiac sphincter or on the stomach.

Openchowski (39) describes and figures inhibitory efferents to the mammalian cardia via the thoracic sympathetic chain of ganglia. Some of these are figured as passing to cardia via the aortic plexus, some as passing via the splanchnic nerves and the gastric plexus.

In 1900 Langley (28) stated that "in the rabbit splanchnic inhibitory fibres for the cardiac sphincter of the stomach arise from the 5th to the 9th thoracic nerves." The experiments on which this conclusion is based are not known to me. A year later Langley (29) showed that epinephrin caused dilatation of the cardia in the rabbit, but this is not a certain evidence of sympathetic inhibitory fibers, especially since in the rabbit inhibitory fibers to the cardia are also present in the vagi. In 1903 Langley (30) made the generalization that "stimulation of the sympathetic produces motor and inhibitory action on the smooth musculature of the gut from the esophagus to the descending colon" (p. 830).

In regard to the innervation of the cardia and lower esophagus in amphibia and reptilia, the following works have come to our notice, since our report on the frog and the turtle (18). Waters (46), by direct inspection, found that stimulation of the III, IV and V spinal nerve roots in curarized frogs caused peristaltic waves and vasoconstriction in the esophagus. Weaker stimulation of these nerves caused vasoconstriction in the esophagus but no peristalsis. Similar motor effects on the toad's esophagus by stimulation of the thoracic sympa-

thetic nerves before their junction with the vagus were later obtained by Bottazzi (4), but subsequently this author reported that the thoracic sympathetic nerves are essentially inhibitory to the esophagus of the toad and the turtle (5). In our previous report we showed that the vagus is the main inhibitory efferent to the turtle's esophagus (18). If the action of epinephrin can be relied on as evidence of sympathetic innervation, this drug induces contraction in the esophagus of the turtle and inhibition (longitudinal musculature) and contraction (circular musculature) of the frog's esophagus (2). On the purely anatomical side there is no lack of evidence of connection between the vagi and stellate and cervical sympathetic ganglia as well as the thoracic sympathetic chain both via the aortic plexus and the splanchnics (20), (22), (38). But these connections may be purely afferents or vasomotor efferents.

The question of sympathetic efferents to the esophagus and cardia is to a certain extent bound up with that of sympathetic efferents to the stomach itself. Most observers agree that stimulation of the splanchnic nerves causes inhibition of the stomach, and that epinephrin induces a parallel inhibition. In 1885 Marcacci (35) (quoted by Luciani) described both motor and inhibitory action on the frog's stomach from stimulation of the sympathetic nerves. One tracing published by Morat (37) shows primary contraction of the dog's stomach on splanchnic stimulation. Spandolini (45) reports both motor and inhibitory action fron splanchnic stimulation in the dog.

The most recent evidence from the action of adrenalin on isolated strips of the mammalian stomach is that reported by Smith (43). This author found that adrenalin causes *contraction* of the cardiac and pyloric sphincters (man, dog, cat, rabbit, guinea pig) *relaxation* of the rest of the stomach (cat, man), while in the rabbit, guinea pig and dog the drug causes contraction in some parts of the stomach and relaxation in other parts.

Page-May (40) ascribed all so-called splanchnic action on the stomach to the escape of the electrical current or stimulus to the diaphragm. There are other possible sources of error. Adrenalin appears to act on the gastric motor mechanism, and it is generally agreed that direct stimulation of the splanchnic nerves causes an increased output of adrenalin. But some of the investigators have eliminated this error by extirpation of the adrenal glands. Again, since the splanchnic nerves carry vasoconstrictor fibers to the stomach and the sphincters, motor action on the stomach may be an indirect one due to temporary

asphyxia from vasoconstriction. But this factor is further complicated by the observations of Hooker (21) that CO_2 stimulates atonic strips, and inhibits rhythmically beating strips from the frog's gut, while as a rule asphyxia appears to have a primary stimulating action on the intact gut both in the frog and the mammal.

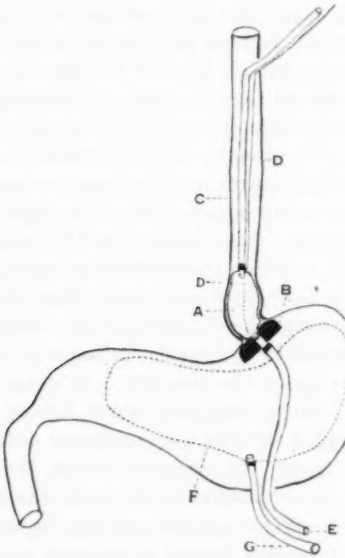

Fig. 1. Diagram to illustrate method of continuous recording of the tonus of the cardia. *A*, (condom 4–6 cm. in length) in cardia and lower esophagus; *B*, rubber plug (2 to 2½ cm. diameter, 1 cm. thick), preventing cardia balloon from being pushed or pulled up into esophagus; *C*, rubber tube (4 mm. diameter), connecting esophageal end of cardia balloon with water manometer via esophageal fistula in neck; *D*, thread passing inside cardia balloon and firmly attached to rubber plug, *B*, for holding balloon, *A*, in cardia; *E*, rubber tube (4 mm. diameter) connecting stomach end of cardia balloon with water manometer through gastric fistula; *F*, balloon (condom, length 10 to 15 cm.) in stomach; *G*, rubber tube connecting gastric balloon with water manometer through gastric fistula.

EXPERIMENTAL METHODS. *1. Recording the contractions of the cardia.* The arrangements devised are shown by diagram in figure 1. The new principles involved in this arrangement are the method of fixing the balloon in the cardia, and the connection of the two ends of the cardia balloon to separate water manometers, thus using the amount

of the respiratory excursion through the cardiac end of the balloon as a measure of the tonus of the cardia. The balloon is made of the most delicate rubber condom, 3 to 5 cm. in length. The cardia guard is made out of a perforated rubber cork, 2 cm. in diameter, for the cat and rabbit, 3 cm. in diameter for large dogs. The end of the guard facing the cardia is made slightly convex and filed smooth. The cardiac end of the balloon and the tube are tied on a glass bead, and this junction pushed into the central perforation of the guard, so that on the cardia side nothing extends beyond the guard, except the delicate balloon. This instrument may be called a cardiameter.

If the tonus or contraction of the cardia exceeds the pressure inside the cardia balloon, it is obvious that the balloon will be completely compressed in the cardia region, and the negative pressure in the chest produced by the inspiratory movements will be registered only through the esophageal end of the cardia balloon; and by raising the pressure in the balloon to a level at which the respiratory movements via the cardiac end are registered synchronously with those from the esophageal end, one has a measure of the strength of the cardia contraction.

In all acute experiments the cardia and gastric balloons are introduced through a fistula in the stomach. A stomach tube is passed through the mouth, the thread and rubber tubing, *C*, *D*, attached, and the apparatus pulled through the esophagus until the cardia guard, *B*, is in place just inside the stomach below the cardia. A small incision is made in the esophagus in the neck, the anchor thread and tubing, *D*, *C*, brought out through this opening, so as to eliminate possible irritation from tube and thread in pharynx and mouth. The anchor thread, *D*, is usually fixed to the tracheal cannula, so that esophageal peristalsis will not move the cardia balloon down into the stomach.

In rabbits and cats, even when under anesthesia, there is little or no chance of the cardia guard (fig. 1, *B*) being pulled up into the esophagus, provided only moderate tension is exerted on the anchor string. If large dogs are used and under anesthesia, this error may occur unless provided against by palpation with the finger through the gastrostomy. During the experiment on dogs and cats vomiting movements may also force the cardia guard into the esophagus, because of excessive intra-abdominal pressure, with dilatation of the cardia. Hence as a routine procedure the exact location of the cardia guard and balloon should be ascertained by palpation at the beginning of the experiment and by post-mortem examination at the end.

Previous investigators of the nervous control of the cardia have relied on direct inspection, the resistance to the forcing of fluids from

the esophagus into the stomach or *vice versa*, the x-ray, and the resistance to the passage of the stomach tube. These methods do not permit continuous recording of the cardia tonus. Furthermore, a column of water under pressure in the esophagus or stomach probably introduces local and long reflex actions involving the cardia.

The only objection I can see to the method used in this investigation is the disturbance that may be caused by mechanical irritation from the apparatus in the cardia and esophagus (especially the inflated balloon). Excessive inflation of the cardia balloon in man causes contraction by central and local reflex action (16), but moderate distention sufficient for these experiments has no such effect either on the normal or the anesthetized animal. Furthermore, we know that similar balloons and tubes in stomach and esophagus of man cause no motor disturbance, or sense of discomfort, except the slight irritation from the rubber tubes in the pharynx and mouth (10), (15). In this work pharyngeal and mouth factor is eliminated by leading the rubber tube and anchor thread out through a neck fistula of the esophagus. V. Mikulicz (34) has shown (man) that mechanical and chemical irritation of the mucosa in the region of the cardia leads to increased tonus or spasm of the cardia, but the smooth rubber guard in the stomach below the cardia does not induce such irritation.

In the acute experiments the opening into the stomach was closed by silk sutures, and the abdomen closed, after fixing the apparatus in place.

The water manometer is not satisfactory for measuring very strong tonus or contractions of the cardia. For such purpose the mercury manometer was used.

2. Anesthesia. Ether was used in all the crucial experiments during the preparation of the animal. To eliminate anesthesia and skeletal muscle contractions we employed decerebration, pithing the entire central nervous system, or the spinal cord below the level of the third cervical segment. In some experiments the chest was opened, and the diaphragm isolated from the esophagus and cardia. A few experiments were made under a combination of ether and curare. Curare is not satisfactory as it induces a prolonged spasm of the cardia.

3. One dog was prepared with a permanent gastrostomy and esophageal fistula in the neck. After complete recovery this animal was used in the study of the relation of gastric tonus to the tonus of the cardia and the effect of ether anesthesia and cocain on the tonus of the cardia. This animal was trained to lie without restraint quietly on a comfortable table for hours at a time.

4. Most of the experiments were made on animals with the stomach empty, but this condition is difficult, if not impossible, to secure in the rabbit. In the rabbit the stomach content was removed through the gastrostomy before introduction of the cardia balloon.

5. In some experiments records were also taken from a second esophageal balloon placed at the level of the heart, that is, in the region composed exclusively of striated musculature. This balloon served as an index of the rate and strength of the respiratory movements. When this method was employed the cardiameter was provided with only one opening (the cardia end), so as to eliminate the disturbance from a second rubber tube in the esophagus. Carotid blood pressure records were usually also run to control the possible passive influence of cardiac and vasomotor changes on the balloon in the cardia.

RESULTS. *1. The relation of the tonus of the cardia to the tonus of the stomach.* Cannon and Washburn (10) showed in man that the lower esophagus exhibits periodic tonus rhythm similar to that of the empty stomach, and they suggest that these gastric and esophageal contractions occur synchronously. Later Carlson and Luckhardt (16) proved (man, dog) that tonus contractions of the lower end of the esophagus run parallel with the hunger contractions of the empty stomach. But the experimental method they used did not permit actual recording of the tonus and contractions of the cardia itself. If the tonus of the cardia is related to the tonus of the stomach in the way that the pyloric sphincter is related to the gastric peristalsis, it is evident that the investigation of the tonus of the cardia cannot be made independent of that of the gastric tonus and contractions.

The intimate relation of the gastric and cardia tonus is shown by the following facts:

a. In the empty stomach (dog) the tonus of the cardia increases and decreases with the tonus of the stomach, and the individual hunger contractions of the empty stomach are accompanied by spasms of the cardia parallel with the strength and duration of the gastric contractions. A typical tracing illustrating these facts is reproduced in figure 2. The increased tonus recorded from the esophageal end of the cardia balloon (fig. 2A) is probably due in part to the contraction of the cardia itself and in part to increased tonus of the entire musculature of the esophagus. In the dog the esophageal musculature down to the cardia is said to be composed of striated musculature. But we have shown (man) that the gastric hunger contractions lead to increased tonus even of the skeletal neuro-muscular mechanism (13).

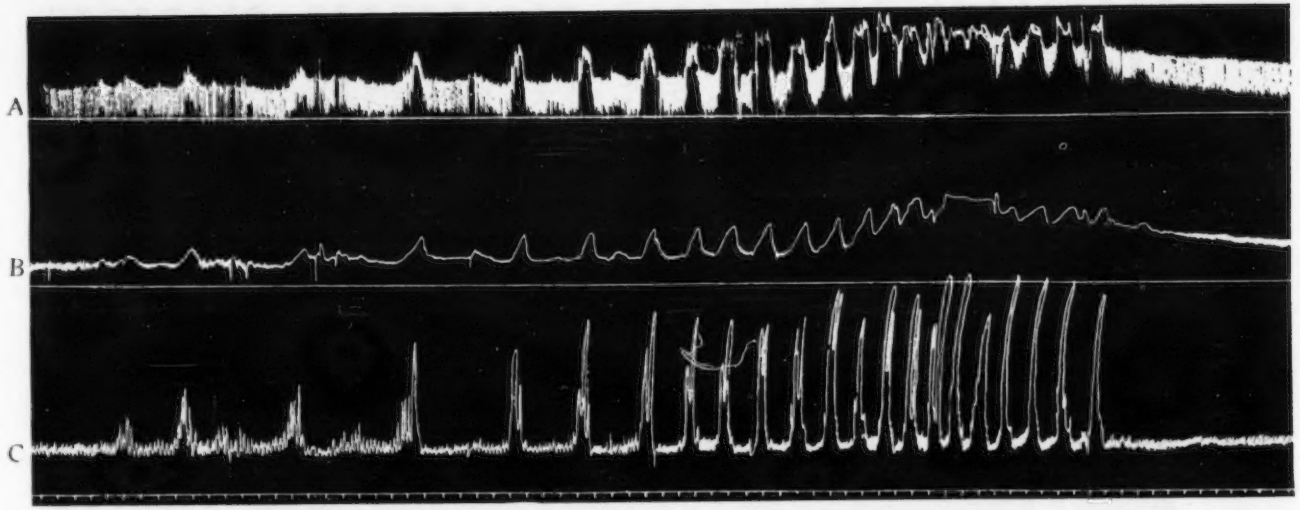

Fig. 2. Dog, gastrostomy and fistula of the esophagus (neck). No anesthesia. Stomach empty; dog lying on right side without restraint. Water manometer tracings of tonus variations in cardia and esophagus parallel with the gastric hunger contractions. A, record from esophageal opening of cardia balloon; B, record from cardia opening of cardia balloon; C, record from balloon in stomach. Time, minutes (total time of record 1 hr. 16 min.). Showing increased tonus of the cardia and lower esophagus parallel with the gastric hunger contractions.*

* The tracings reproduced in this article are reduced to $\frac{1}{2}$ to $\frac{1}{4}$ of the original.

We have not been able to determine whether cardia contractions precede and initiate the gastric contractions or *vice versa*. By the present method the two series appear to run parallel. There are three possible correlations; *1*, both may be initiated by the same central mechanism (depression of inhibitory and initiation of motor impulses via the vagi and splanchnic nerves; *2*, initiation of the gastric contractions by the cardia as the primary pace maker; *3*, initiation of the cardia contractions reflexly by the contractions of the stomach. In fact, all three factors may be involved, and the rôle of either one must be determined by another line of investigation.

b. Anesthesia depresses the tonus of the cardia parallel with that of the stomach (fig. 3). All the experiments on this phase were done on the dog with the permanent gastrostomy and esophageal fistula. Light ether anesthesia may lower the cardia tonus (empty stomach) from 10 to 30 cm. of water to 1 to 3 cm. of water. These results are invariable, at least in the dog, and seem to parallel the abolition of clinical cardiospasm by light anesthesia (retention of the lid reflex). If light anesthesia is changed to very deep anesthesia there usually follows (dog, cat) an increase in the cardia and gastric tonus. This is probably due to partial asphyxia. These results are in line with those reported by Kelling (23).

The mechanism of this tonus depression by light anesthesia is not clear. If it is due to failure of central tonus innervation, it should be absent in animals with the cardia and stomach isolated from the central nervous system, if this operation is possible. The practical significance of the fact itself in the present investigation is this. Since most if not all direct stimulation of the cardia-gastric nerves must be done under anesthesia, or after operative procedures rendering the animal more abnormal than the state of light anesthesia itself, we are obviously working on a *hypotonic* and to that extent abnormal mechanism. The state of tonus of the peripheral mechanism may alter the quality and quantity of the response to nerve stimulation.

c. The tonus of the cardia, in case the stomach is empty and relatively quiescent (absence of hunger contractions) *is usually much feebler than when the stomach is in the state of digestion.* This is true even in crucial experiments under general anesthesia. Cannon (9) has reported some observations tending to show that acid in the stomach increases the tonus of the cardia reflexly. Assuming for the present that acid in the stomach is one factor in the increased tonus of the cardia during digestion, the following facts show that other factors are involved:

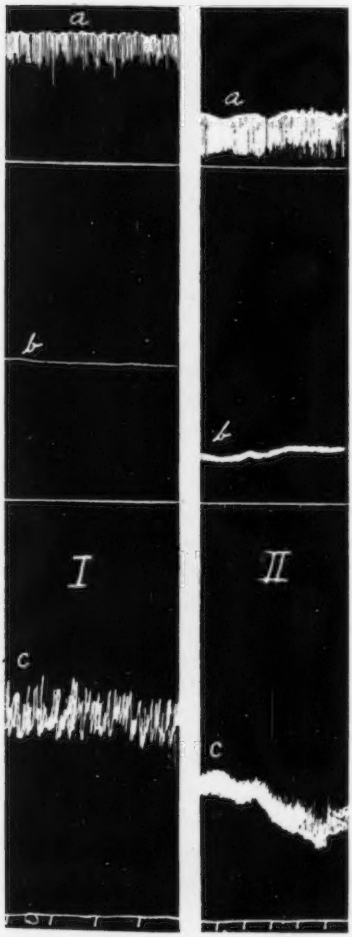

Fig. 3. Dog with permanent gastrostomy and esophageal fistula (neck). I, No anesthesia; II, light ether anesthesia (retention of lid reflex): *a*, record from esophageal end of cardia balloon; *b*, record from cardia end of cardia balloon; *c*, record from balloon in stomach. Showing depression of tonus of cardia by anesthesia. Time, minutes.

1. The hypertonus of the cardia during gastric digestion persists to a marked degree under general ether anesthesia, even after the food and chyme has been removed from the stomach, the stomach washed out with water at body temperature, or rendered alkaline by Na_2CO_3.

2. Introduction of 50 to 100 cc. of 0.4 per cent HCl into the empty and quiescent stomach does not increase the tonus of the cardia comparable to that found in the digesting stomach. This applies, so far, only to the cat and dog, as rabbits with completely empty or fasting stomachs have not been worked on.

This question will be dealt with further in reporting on the reflex control of the cardia.

The foregoing facts suffice to show that even in the investigation of the relatively simple problem of response of the cardia to direct stimulation of the extrinsic cardia efferents, it is necessary to record the gastric tonus, control the state of gastric digestion, and to recognize that under general anesthesia the tonus of the cardia is usually so depressed that the response of the cardia may be altered, quantitatively and qualitatively.

2. The effect of section of the vagi nerves. In the majority of experiments under ether anesthesia (dog, cat, rabbit) section of both vagi nerves in the neck produces spasms of the cardia, lasting from 3 to 15 minutes, when the tonus returns practically to its original level. This temporary cardiospasm seems to be due mainly to mechanical stimulation of motor fibers in the vagi, since similar cardiospasms in these preparations may be induced by electrical or mechanical stimulation of the peripheral end of the cut vagus. If the cardia is in strong tonus at the time of the vagi section the cardiospasm may be preceded by a temporary (1 to 2 minutes) inhibition of the cardia tonus.

Similar temporary cardiospasms follow section of both vagi in decerebrated cats. It should be noted, however, that our experiments were made within a few (2 to 4) hours after decerebration. In animals kept for several days after decerebration section of the vagi may have a more prolonged effect on the cardia. In cats and dogs decerebration (under ether) depresses the tonus of the cardia, at least for several hours.

In five out of forty cats and in three out of seven rabbits, section of both vagi in the neck induced spasm of the cardia that lasted for several hours, or throughout the experiment. In four of the cats the vagi section caused a parallel increase in the gastric tonus. A record illustrating these results for the cat is reproduced in figure 4.

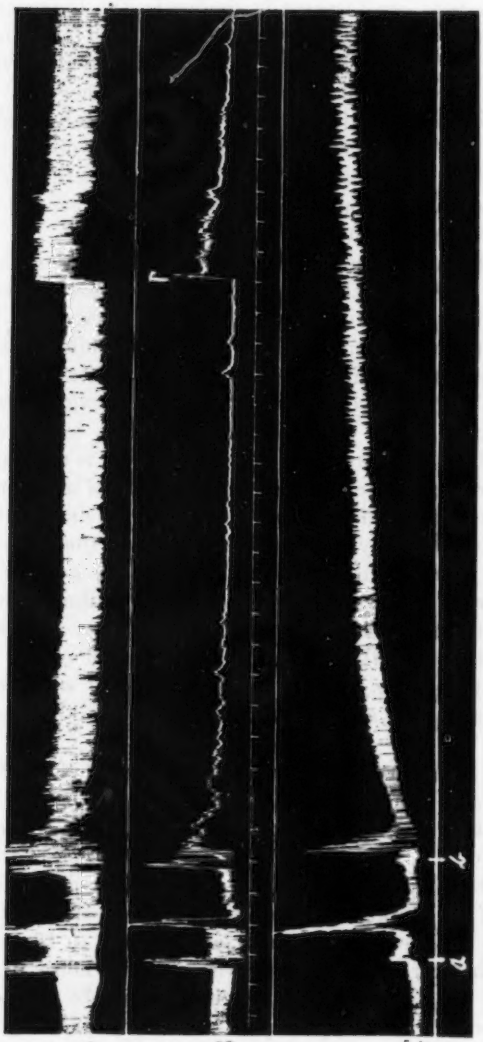

Fig. 4. Cat. Light ether anesthesia. Water manometer record. A, esophageal opening of cardia balloon; B, cardia opening of cardia balloon; C, balloon in stomach; *a*, section of right vagus; *b*, section of left vagus; *p*, increase of pressure in cardia balloon. Showing prolonged (3 hours +) hypertonus of cardia (and stomach) following section of the vagi. Time, minutes.

This more permanent cardiospasm is similar to the hypertonus of the esophagus of the turtle and the lungs of the amphibia following vagi section (17), (18), (41). Bercovitz and Rogers (3) have noted similar hypertonus in the turtle's stomach on section of the vagi nerves. There are indications that after section of both vagi aseptically and with recovery of the animals, there may follow a hypertonus of the cardia, lasting for 3 or 4 days, but the evidence on this point is rather indirect. The literature is reviewed in our paper on the nervous control of the esophagus in the turtle and the frog (18).

In the case of the esophagus of the turtle and the lung of the frog Doctor Luckhardt and the author showed that permanent hypertonus is due to the removal of the tonic inhibitory control exercised by the vagi over the peripheral automatism, a mechanism similar to the acceleration of the heart following section of the vagi nerves. Is this explanation tenable for the cardiospasm following vagi section in mammals? The vagi nerves contain inhibitory fibers to the cardia, but if these inhibitory fibers exhibit tonic action, why is not vagi section followed by prolonged cardiospasm in all, instead of in exceptional experiments? The varying degrees and effects of the anesthetic may be a factor in this discrepancy. But before we accept the explanation of a tonic inhibitory vagus action on the cardia as the sole factor in the cardiospasm following section of the vagi, it must be shown that the mechanical irritation from section of motor paths may not last for hours or days, and further, that the motor innervation of the cardia via the sympathetic system plays no rôle. We shall see presently that the mammalian cardia actually receives motor fibers via the sympathetic system.

The possibility that mechanical irritation of motor efferents may last for a considerable time is not an idle speculation. It was shown by Doctor Luckhardt and the author that this is the main factor in the hypertonicity of the frog's esophagus following section of the vagi or destruction of the peripheral end of the cut vagi or destruction of the brain (18). If the irritation of the peripheral end of the cut vagi for 5 seconds may induce a cardiospasm lasting for 5 to 15 minutes, why may not such after-effects extend over as many hours or days? Lesion or ablation of the motor cerebral cortex or a lobe of the cerebellum (also motor) induces temporary (2 to 3 days) spasticity of the skeletal motor mechanism. One explanation advanced for this phenomenon is continuous mechanical and chemical irritation of the cut motor paths. This is probably not an adequate explanation. We

may point out, however, a further parallel between the effects of cerebellar ablation on the skeletal motor system, and of vagi section on the cardio-gastric motor mechanism. In the former case the temporary effects are hypertonicity, the permanent effects hypotonicity. Similarly, section of both vagi seems to render the stomach more or less hypotonic as a permanent effect (7), (14), following a more transitory phase of hypertonicity of the lower esophagus, cardia and stomach, as noted above. This temporary hypertonicity of the stomach region in the mammal following section of the vagi must be studied more extensively and by more physiological methods.

3. The motor and inhibitory action of the vagi on the lower esophagus and cardia. In dog, cat and rabbit under light ether anesthesia, with or without curare, or with the central nervous system destroyed, stimulation of the peripheral end of the sectioned vagus (left or right) produces both inhibition and contraction of the cardia. This applies also to the lower end of the esophagus in the case of the cat. This is merely confirmatory of the earlier reports of Openchowski (39), Langley (27), Meltzer and Auer (36), Rogers and Bercovitz (42) on mammals, and of Carlson and Luckhardt on the turtle (18). The inhibition is most readily obtained from vagus stimulation if the cardia is hypertonic, motor effects if the cardia is relatively atonic at the time of the stimulation (fig. 5).

It would appear that both the motor and inhibitory action is due to the vagi efferents, because they may be secured by stimulation of the vagi near the cranial exit, and stimulation of the cervical sympathetic nerves above the stellate ganglion seems to be without cardia-gastric influence; and we have no evidence that cardia-gastric efferents join the vagus system from the cervical nerve roots.

The efferent action of the vagi on that part of the esophagus composed solely or mainly of striated musculature appears to be purely motor. At least we have never observed any inhibitory action. Vagus inhibitory (and sympathetic motor and inhibitory) action thus seems to be lost with the disappearance of the Auerbach's plexus, and the change of the musculature to striped variety. But to the lower esophagus in the cat, and to the cardia and the stomach in the dog, cat and rabbit, the vagi serve both as motor and inhibitory nerves.

4. The action of the splanchnic nerves on the cardia and lower esophagus. In cats and dogs under light ether anesthesia, with or without curare, or with the spinal cord or with brain and spinal cord pithed, mechanical or electrical stimulation of the peripheral end of the splanchnic

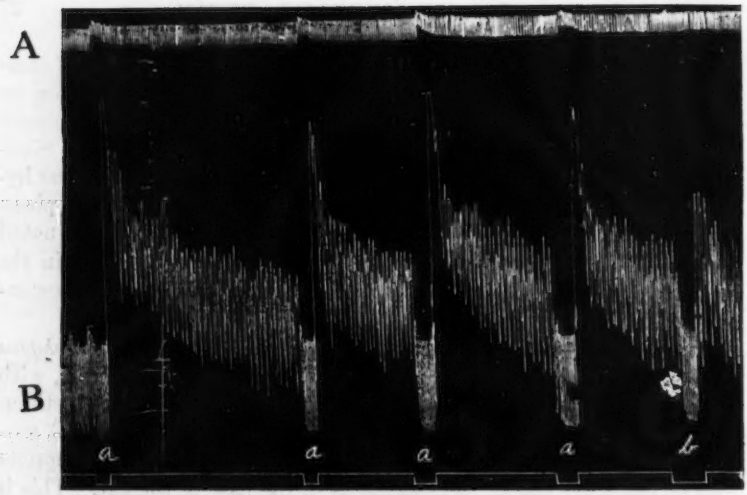

Fig. 5. Cat. Ether, curare, artificial respiration. Left vagus nerve sectioned. Water manometer tracings. A, from esophageal balloon at level of heart. B, in lower esophagus and cardia; *a-a*, tetanization of peripheral end of left vagus; *b*, ditto, central end of left vagus. Showing direct and reflex inhibition of the cardia via the vagi.

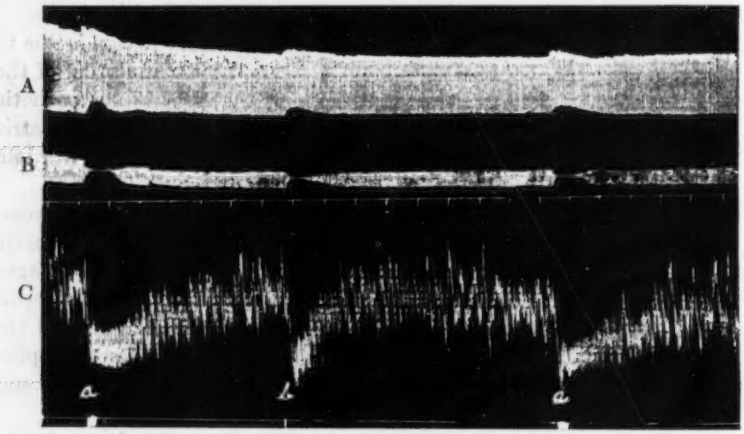

Fig. 6. Cat. Ether. Brain and spinal cord pithed, both adrenals extirpated. Artificial respiration. Water manometer records, A, esophageal opening of cardia balloon; B, cardia opening of cardia balloon; C, balloon in stomach. *a-a*, tetanization of peripheral end of left splanchnic nerve; *b*, intravenous injection of 1 cc. 1/100,000 adrenalin. Showing opposite action of adrenalin and of splanchnic stimulation on cardia and stomach. Time, minutes.

nerves (major and minor) usually causes contraction of the cardia and the lower esophagus (cat) (figs. 6, 7). The stimulation (5 to 10 seconds) may induce a spasm of the cardia lasting for 5 or 10 minutes. In dogs or cats under ether anesthesia, with or without section of the vagi in the neck, section of the splanchnic nerves causes contraction of the cardia or a cardiospasm lasting for 3 to 10 minutes. In similar preparations, with the vagi sectioned, pithing the spinal cord induces a prolonged spasm of the cardia (fig. 9).

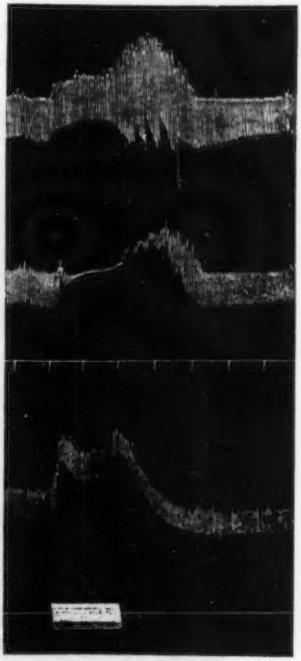

Fig. 7. Cat; ether anesthesia. Water manometer records of gastric and esophagus-cardia contractions. A, esophageal opening of cardia balloon; B, cardia opening of cardia balloon; C, gastric balloon. Signal, tetanization of peripheral end of the left splanchnic nerve. Showing contraction of stomach and cardia on splanchnic stimulation. Time, minutes.

In dogs under anesthesia we have so far never obtained inhibition of the cardia from splanchnic stimulation, and we worked on cats for more than three months, using over thirty animals, before instances of inhibition of the cardia from peripheral splanchnic stimulation were seen (fig. 8). We had reached the conclusion, for the cat and dog, that the splanchnic efferent nerves have purely motor action on the cardia and esophagus. The main reason for the prevailing motor action of the splanchnic stimulation in the cat and dog is probably the predominant hypotonus of the cardia due to the anesthesia, decerebration, etc. At any rate, in the few cases in cats that splanchnic stimulation caused inhibition of the cardia, the cardia was in very marked tonus at the time of the stimulation.

In rabbits, under general anesthesia, stimulation of the peripheral end of the splanchnic nerves has so far (7 animals) yielded only inhibition of the cardia. But the inhibitory action of the splanchnics on the cardia was obtained in all the experiments.

We do not conclude that in the rabbit motor efferents to the cardia are absent in the splanchnic nerves. We are inclined to ascribe the prevailing inhibitory action to the marked tonus of the cardia in the rabbit.

Before we may conclude from these experiments that splanchnic efferents (motor and inhibitory) to the cardia and esophagus in mam-

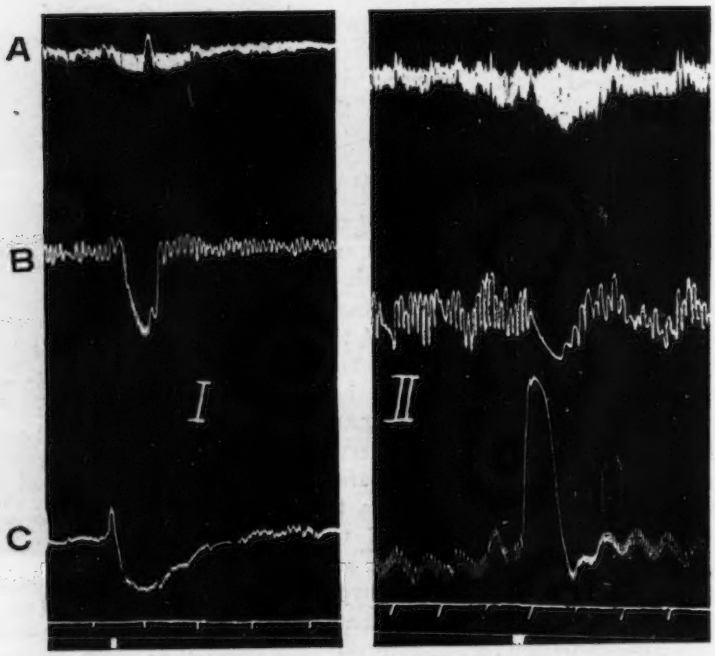

Fig. 8. Cat. Ether. I. Vagi sectioned. II. Vagi and splanchnic nerves sectioned. Both adrenals extirpated. A, Water manometer record from balloon at level of heart; B, ditto from balloon in cardia; C, Carotid blood pressure; I, signal, = intravenous injection of 1 cc. 1/100,000 adrenalin; II, signal, tetanization of peripheral end of both splanchnic nerves. Showing inhibition of hypertonic cardia by adrenalin and splanchnic stimulation. Time, minutes.

mals are demonstrated, it must be shown that the effects on the cardia are not due to contractions of the diaphragm; to mechanical factors following contraction or relaxation of the stomach; to increased output of epinephrin; or to local vasoconstriction in the cardia and esophagus.

According to Page-May, the motor or inhibitory effects on the stomach described by previous investigators as due to stimulation of the splanchnic efferents are purely artifacts from contraction of the diaphragm because of escape of the electrical current to the corresponding crus. Every investigator in this field will admit that this error may vitiate

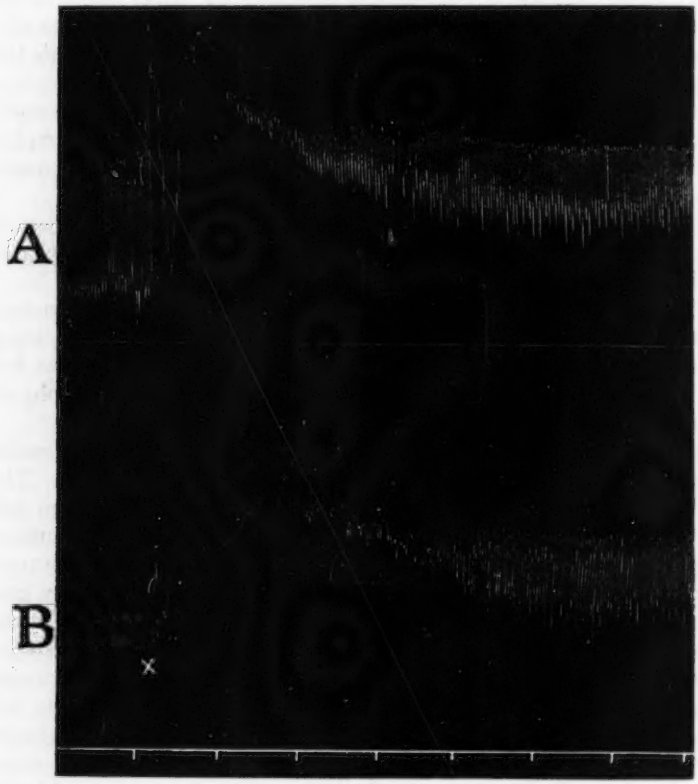

Fig. 9. Cat. Ether. Vagi sectioned; spinal cord sectioned at level of 3rd cervical; artificial respiration. Water manometer records of balloon at lower esophagus and cardia. A, esophageal opening of balloon; B, cardia opening of balloon. *X*, Pithing spinal cord. Showing prolonged contraction of lower esophagus and cardia on destruction of spinal cord (mechanical stimulation of splanchnic motor fibers). Time, minutes.

the results unless special care is taken to prevent the escape of the current when the electrical stimulus is used. The objection of Page-May does not apply when the splanchnics are stimulated mechanically. If contraction of the diaphragm accounts for the apparent splanchnic influence on the stomach and cardia, this action on the stomach, at least, should always be an apparent contraction. This is not the case.

We have eliminated Page-May's objection by direct experiments on the cat. It is possible to dissect away the greater part of the diaphragm connections with the esophagus without interfering with the splanchnic action on the cardia and lower esophagus. In such preparations the contraction of the cardia and lower 2 to 4 cm. of the esophagus on splanchnic stimulation can still be made out by the graphic method, by direct inspection, or by the increased pressure on the finger pushed into the esophagus from the gastric side.

The epinephrin factor was eliminated (cat, dog) by extirpation of both adrenal glands. This operation does not abolish the splanchnic action on the cardia.

The indirect effect of local vasoconstriction is less readily eliminated. But we have succeeded in a number of experiments (cats) in obtaining typical splanchnic action on the cardia and lower esophagus for a few minutes after excision of the heart. This eliminates the asphyxia factor from local vasoconstriction.

There remains the possibility of mechanical effects on the cardia balloon from contraction or relaxation of the stomach itself. The action of the splanchnic efferents on the gastric motor mechanism will be taken up in a separate paragraph. In this connection it suffices to point out that in dogs and cats under anesthesia, the cardia contraction following splanchnic stimulation is usually accompanied by gastric inhibition, but sometimes by gastric contraction (figs. 6, 7); in the rabbit the cardia inhibition is usually accompanied by gastric contraction, but it may also be accompanied by gastric inhibition. Obviously purely mechanical factors from changes in the gastric tonus do not account for the changes in the tonus of the cardia and lower esophagus (cat) on splanchnic stimulation. We therefore consider the presence of splanchnic efferents (motor and inhibitory) to the cardia and lower esophagus in mammals as demonstrated.

As additional evidence we may note that with the splanchnic nerves intact and the vagi cut, pithing the spinal cord induces a prolonged contraction or spasm of the cardia and esophagus in the cat (fig. 9). This is in line with the prevailing motor action of splanchnics in this animal when under ether anesthesia.

5. Is there a sympathetic nerve supply to the lower esophagus and cardia other than that via the splanchnics? Anatomical basis for such innervation via the aortic plexus have been reported by Openchowski (39) and especially by Müller (38). We have tested this possibility experimentally in cats in the following way: Under ether anesthesia and artificial respiration after section of the splanchnic nerves on both sides, pithing the spinal cord, ligation and excision of the left lung, the thoracic sympathetic chain on the left side was stimulated at different levels up to and including the stellate ganglion, with and without section of the vagi in the neck. The pithing of the thoracic spinal cord is necessary in these experiments to eliminate reflex action (respiratory and skeletal), as the thoracic sympathetic trunk appears to contain numerous afferent fibers.

In some of these experiments contraction of the cardia was obtained on electrical stimulation of the upper thoracic sympathetic trunk, including the stellate ganglion. In other experiments the stimulation had no effect on the cardia and lower esophagus. In no case did stimulation of the sympathetic cephalad to the stellate ganglion have any effect on the cardia, even when the vagi were intact. We do not know how to interpret these inconstant results. There may be individual variations in the pathways of the sympathetic efferents to the cardia and lower esophagus. Or, our positive results are due to escape of the electrical current. In no case did we obtain contraction of the cardia from mechanical stimulation of the thoracic sympathetic after section of the splanchnics on the same side. But it must also be noted that in some animals mechanical stimulation of the splanchnic nerve also failed to affect the cardia, despite the fact that these nerves do contain motor efferents to the cardia.

6. The action of epinephrin on the cardia and lower esophagus. We were interested in the action of epinephrin on the cardia primarily for the purpose of determining the epinephrin rôle in the cardia effects of splanchnic stimulation in the preparations with the adrenal gland intact. In all our experiments on dogs under ether anesthesia intravenous injection of epinephrin causes contraction of the cardia. This is true in the weakest concentration that had a demonstrable motor action (1 cc. 1/400,000) up to the strongest concentration used (1/50,000). Many experiments were made on the dog with the permanent gastrostomy and esophageal fistula. In this dog epinephrin intravenously invariably caused dilatation of the cardia (fig. 10).

The explanation for the opposite action of epinephrin on the cardia when no anesthesia is used is not clear. We have already shown that anesthesia depresses the tonus of the cardia. It may be that with cardia tonus low the pressor action of epinephrin comes to the front, while with cardia tonus high the depressor action of the drug predominates. We have so far noted only inhibitory action of this drug on the gastric tonus in the dog.

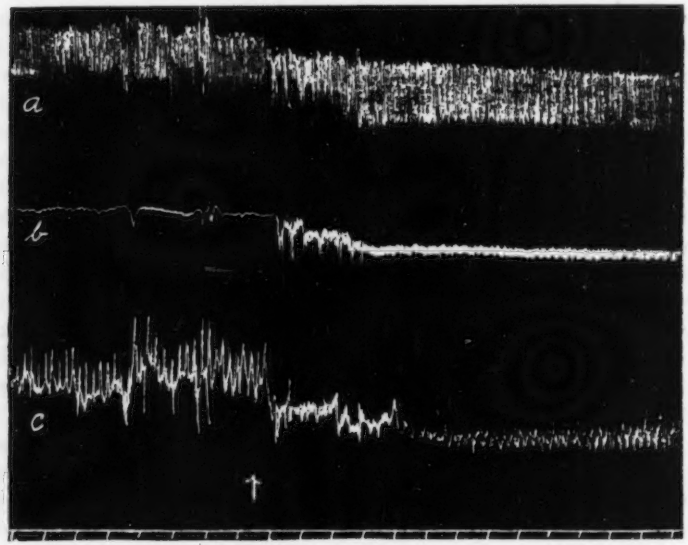

Fig. 10. Dog with permanent gastrostomy and esophageal fistula (Neck). Water manometer tracings; *a*, esophageal opening of cardia balloon; *b*, cardia opening of cardia balloon; *c*, balloon in stomach; ↑, intravenous injection of 2 cc. 1/100,000 adrenalin. Showing inhibition of the cardia and stomach by adrenalin. Time, minutes.

In cats under anesthesia the prevailing action of epinephrin is motor on the cardia and lower esophagus, and inhibitory on the stomach (fig. 6, 7). In a few experiments epinephrin caused inhibition of the cardia and lower esophagus (fig 8). These preparations showed exceptionally high cardia tonus.

In rabbits under anesthesia epinephrin has so far produced only inhibition of the cardia, parallel with contractions of the stomach (fig. 9).

The prevailing view that epinephrin depresses the motor mechanism of the gut is therefore not tenable. The primary action of this drug on the gut seems to depend on the tonus of the motor mechanism rather than on the dominance of inhibitory over motor sympathetic innervation.

We may recall that Langley reported epinephrin depression of the rabbit's cardia; while using isolated strips, Smith obtained only motor action from the drug on the cardia of man, monkey, dogs, cats and guinea pigs. These apparent discrepancies of epinephrin action are probably accounted for mainly by the variation in the tonus factor. At least in the dog and cat, epinephrin depresses the cardia when the tonus is high, and stimulates the cardia when the tonus is low. It is evident that we must recognize a double action of epinephrin on the gut similar to that on the vascular motor mechanism. The observations of Smith on isolated strips from the mammalian stomach showed both motor and inhibitory effects of epinephrin. Similar results were obtained by Bercovitz on the frog's esophagus, while in the turtle epinephrin appears to have a uniformly motor action both in the intact animal and on isolated strips (circular and longitudinal systems).

7. The action of atropin on the vagi and sympathetic efferents to the cardia. The abolition of the vagus action on the stomach motor mechanism in mammals by atropin seems to be a well-established fact. The conflicting results reported in the literature are probably due to the varying quantities of the drug used. On the whole, the vagi efferents to the heart seem to be more readily paralyzed by atropin than the vagi efferents to the stomach.

This atropin action on the vagi efferents to the stomach includes the cardia. Langley was able to show, in the rabbit, that the vagus inhibitory mechanism of the cardia is slightly more resistant to atropin than the motor mechanism. But both systems are readily paralyzed by the drug. It is also well known that some of the sympathetic efferents are more resistant to atropin than is the vagus system. This raises the question whether atropin abolishes the splanchnic nerve action on the cardia and lower esophagus.

All our experiments on this point were made on cats under ether anesthesia. Intravenous injection of 1 or 2 mgm. atropin sulphate abolishes both vagi and splanchnic action on the cardia and lower esophagus, practically simultaneously. There is no significant difference in the action of this drug on the two systems of efferent nerves to the cardia and lower esophagus.

8. The action of splanchnic nerves on the stomach. Our observations on this point were incidental to the work on the cardia and lower esophagus. In the dog and cat under anesthesia and with the stomach empty, the prevailing action of the splanchnics on the gastric motor mechanism is inhibitory (fig. 6). But in many instances distinct contraction of the stomach was obtained under conditions that eliminated escape of the stimulus to the diaphragm or the stomach itself (fig. 7). In two experiments (dog) the gastric contraction was evident from direct inspection. The contraction seemed to be confined to the antrum. Smith reported contractions from epinephrin in strips from the fundus and body of the dog's stomach.

In rabbits under anesthesia splanchnic stimulation usually induces contraction of the stomach (fig. 9). Inhibition of the stomach was seen, but less frequently. We did not expose the stomach for direct inspection or otherwise determine the region of the stomach concerned in the contraction. Smith reported stimulating action by epinephrin on fundic strips from the rabbit stomach.

The contraction of the stomach noted by us on stimulation of the splanchnic nerves was never as great as that which could be induced by stimulation of the vagi. But it seems clear that the prevailing view of a qualitatively opposite action of the vagi and sympathetic on the stomach is no longer tenable. Both systems produce motor and inhibitory effects on the stomach as well as on the cardia. This being the case, the action of epinephrin on the cardia and the stomach parallels the action of the sympathetic as well as that of the vagus nerves. In this field the epinephrin action is therefore of no value as evidence of sympathetic innervation.

In three experiments (cat) we noted initiation of hypertonus and peristaltic contractions of the pylorus by splanchnic stimulation. Contraction of the duodenum sphincter can practically always be seen on splanchnic stimulation, (cat, dog, rabbit). These incidental observations are noted because the motor innervation of the small intestine via the sympathetic system is not generally recognized.

Discussion. This and other studies on the visceral nervous system in our laboratory have led us to question the adequacy and utility of the prevailing view as to the structure and function of the autonomic system. At present the visceral efferents in the vagi, the splanchnics, etc., with their peripheral ganglionic connections, are considered as practically analogous to the motor efferents of the skeletal muscles. Langley introduced the conception of the "axon reflex," and denies

the existence of true reflexes in the visceral system apart from central connections. This view has recently been emphasized by Alvarez as a negative proposition in support of his theory of a gradient of purely muscular automatism. The apparently fixed reactions of the heart to vagi and sympathetic stimulations seemed to have fixed the physiological point of view for the rest of the viscera. Hence the formulation of problems like these: Does the vagus send motor fibers to the small intestine? Do the vagi innervate the large bowel? Do the splanchnic nerves carry motor fibers to small intestines, etc.

Granted that in some parts of the viscera the relation of visceral efferents to the end organs may be as simple or direct as the relation of the pyramidal tract to the skeletal motor neurones in the spinal cord, it does not follow that all visceral efferents have equally simple peripheral relations. It seems to me that at least some of the visceral nervous relations can be more adequately investigated on the working hypothesis that *the visceral nerves are complex association paths connecting cerebro-spinal and visceral reflex centers, rather than relatively simple nerves like the skeletal efferents.*

There is very little similarity in the effects of vagi and splanchnic stimulation on the stomach, and the effects of sciatic stimulation on the leg musculature; but there is much similarity between the former effect and the influence of stimulation of the cerebellar efferents on the skeletal motor system. In both cases we have either motor or inhibitory effects from the same point of stimulation, depending on the tonus state of the peripheral mechanism. The variability in the response of the gut to vagi and splanchnic stimulation is analogous to the delay and variability in complex reflex responses in the spinal cord. We do not ask: do the sciatic afferents innervate the front legs? The physiological question is: Under what conditions (central and peripheral) do impulses (excitatory and inhibitory) via the sciatic afferents reach the final motor neurones of the front legs?

Is it reasonable to suppose that masses of associated nerve cells, in all essentials histologically similar to nerve cell groups in the brain and spinal cord, are devoid of reflex function, simply because of location, especially in view of the fact that the entire nervous coördination in many animal groups (e.g., the molluscs) is carried out in a nervous system just as diffuse as the visceral nervous system in the mammal? It is probable that the general acceptance of the visceral nervous system as relatively simple conduction paths or peripheral nerves devoid of reflex function has been aided by the theory of muscular automatism

and coördination totally independent of local nervous action. This theory (myogenic theory) has received its greatest attention in connection with the heart, but it is being applied more or less uncritically to the other visceral motor mechanisms such as the gut, the uterus, the ureters, etc. Alvarez (1) endeavors to explain the motor phenomena of the gut on the basis of a polarized muscular automatism or gradient. The most extreme instance of uncritical application of the muscular automatism theory is the recent work of Carey (11) on the urinary bladder. Carey produced a fistula of the urinary bladder in one 4-weeks old pup, and for a period of 50 days the bladder was subjected for varying periods to abnormal internal pressure by forcing boric acid solution in through the fistula. Under these high hydrostatic pressures in the bladder the dog finally died (of uremia). While the dog was yet in good condition, as reported by the author, the bladder showed rhythmical beats like the heart, and he concludes from this one experiment that the high pressure in the bladder had *developed in the bladder musculature the property of rhythmicity.* The bladder, sphincters and urethra were left in their normal connections with their reflex centers in the spinal cord. It is not surprising that the local nervous tissues in the urinary tract are ignored in the genesis of rhythmicity of the bladder, for that is a common error, but to disregard or fail to control the central reflex mechanism of the bladder and sphincters is surprising, to say the least! In the embryo the development of the local nervous tissues and the development of rhythmic activity seem to run parallel (47).

But I would be the last one to deny correlation of differentiation in the muscular and nervous elements of rhythmic neuro-muscular mechanisms. Denial of or ignoring either factor in absence of demonstrated facts retards progress in its failure to suggest new working hypotheses and new methods of experimental attack. It was shown by me years ago that even in the conduction rate in the motor nerves and contraction rate in the muscle there is a direct relation in corresponding motor mechanism (12). And in the diaphragm muscle there appears to be a differentiation in direction of rhythmicity greater than that in the leg muscles (31), although the normal respiratory movements are automatic and reflex acts. According to Prakken (48), the excised striped musculature of the cat's esophagus contracts rhythmically in oxygenated Ringer's solution, but such a mechanism would not serve deglutition; hence it is not physiological.

The fact that at least under present experimental conditions the stomach (including the cardiac and pyloric sphincters and the lower esophagus) and the small intestines are under motor and inhibitory control both of the vagi and the splanchnic efferents renders untenable the view of physiological antagonism of the vagi and sympathetic for this part of the viscera. This has been recently emphasized, especially by Spandolini (44). It also calls for a redefinition of the terms "vagotonia" and "sympatheticotonia" as used in clinical medicine.

SUMMARY

1. A method is described permitting continuous recording of the tonus and contractions of the cardia in acute experiments and in animals provided with a permanent gastrostomy and esophageal fistula.

2. Light anesthesia depresses the tonus of the cardia; deep anesthesia increases the tonus of the cardia, probably through asphyxia.

3. In the animal without anesthesia the tonus of the cardia runs parallel with that of the stomach, but the causal relation between the two is not determined.

4. When gastric digestion is in progress the tonus of the cardia is high, even under anesthesia, and this hypertonus persists after removing the food from the stomach, washing the stomach cavity with water at body temperature, or rendering the stomach contents alkaline; 0.4 per cent HCl in the stomach does not increase the tonus of the cardia parallel to that found in the digesting stomach.

5. Under anesthesia, section of both vagi induces a cardiospasm lasting usually only 3 to 15 minutes. In exceptional cases (cat, rabbit) the cardiospasm may last for several hours.

6. The vagi contain both motor and inhibitory efferents to the cardia and the lower esophagus (cat). On stimulation of the peripheral vagus the motor action prevails if the cardia is in low tone, the inhibitory action prevails if the cardia is in strong tone at the time of stimulation.

7. The splanchnic nerves carry both motor and inhibitory efferents to the cardia and esophagus in the cat. In dogs only motor action, in the rabbit only inhibitory action was demonstrated. These variations are probably due to the condition of the cardia, since it was noted in cats that inhibitory splanchnic action on the cardia and esophagus was obtained only when these organs were in strong tonus. Atropin abolishes both vagus and splanchnic action on the cardia and lower esophagus.

8. Epinephrin has motor and inhibitory action on the cardia and esophagus in the cat and dog. In the rabbit inhibitory action only was demonstrated. The motor action of the drug appears to prevail when the cardia is hypotonic; the inhibitory action when the cardia is hypertonic.

9. On the stomach epinephrin has a predominantly inhibitory action in the cat and the dog, a predominantly motor action in the rabbit.

10. The splanchnic nerves, like the vagi, carry both motor and inhibitory efferents to the stomach. Splanchnic motor action on the duodenum was also noted.

11. As a working hypothesis, the view is advanced that the action of visceral efferents (sympathetic and autonomic), at least on some visceral motor mechanisms, are association or reflex responses, and not simple peripheral responses like that of the skeletal muscles on stimulation of the pyramidal tract. That is, the visceral efferent nerve fibers are in reality afferents to the local but diffuse reflex nervous centers in the visceral organs. The prevailing view of antagonistic action of the vagus and the splanchnic systems is not tenable for the cardia and the stomach.

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A BLOOD ANTICOAGULANT OBTAINED FROM BODY TISSUES; ITS CHEMICAL NATURE AND ITS MANNER OF ACTION

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In our recent work on "tissue fibrinogen" and its action in blood clotting (1) we noted that the protein fraction of the "tissue fibrinogen" when taken alone acted as a very active anticoagulant. We will now describe in detail the method of preparing this substance, its chemical nature so far as we are able and its manner of action in rendering the blood non-coagulable both in vivo and in vitro.

Other anticoagulants of a protein nature that have been recognized are hirudin and the inhibitory substance present in "peptone plasma" of dogs. Hirudin, the anticoagulant of leech extract, is supposed to be protein in nature, a thermo-stable albuminose, but the mechanism by which it prevents blood clotting has never been discovered. Just what there is in its constitution that enables it so effectually to inhibit fibrin formation is still an open question. Antithrombin of peptone plasma is likewise supposed to be protein in nature and thermolabile. Again the chemistry and exact manner of action are unknown. Howell (2) has shown that either of these two bodies (or he suggests they may be one and the same) is capable of preventing the activation of prothrombin to thrombin, or, if thrombin be already formed, its reaction with fibrinogen will be inhibited. He therefore termed the substance "antithrombin." He found its action to be neutralized by tissue extracts or by cephalin. He presented, however, no explanation of the mechanism of its antithrombic activity or of the chemical characteristics responsible for this activity.

A coagulation inhibiting substance has been obtained from the uterine and intestinal mucosa of mammals, the vasodilatin of Popielski (3), and another has been discovered in the anterior part of the body of the *anchylostoma caninum* by Loeb and Fleischer (4). The chemistry

and manner of action of neither of these substances are understood at present.

Doyon and Sarvonat (5) have done a large amount of work in attempting to isolate an anticoagulant from normal liver tissue and have finally arrived at the conclusion that the inhibitory substance in their preparations is nucleic acid. It is difficult to see how nucleic acid could prevent clotting in any way, unless by acting as a decalcifying agent. The concentration in which they find it active is about such as would be required to bind the calcium of the blood. Thus they state that a concentration of 0.5 per cent sodium nucleate in blood will prevent coagulation just as will the same amount of potassium oxalate. Considering the molecular weight of sodium nucleate as about 1400 and supposing that a molecule of calcium nucleate would contain only one calcium atom, then the 0.5 per cent concentration of nucleic acid would be capable of binding calcium to the extent of 14 mgm. per 100 cc., or more than the amount normally present in blood. However, they state that it does not interfere with the clotting of milk by rennin and does inhibit the clotting of fibrinogen or oxalate plasma by thrombin, so it would seem that it is not acting as a decalcifying agent but possesses some unknown manner of action.

The anti-prothrombin or heparin obtained by Howell (6) from liver and heart tissue is a phospholipin. He considers its inhibitory action as being due to its ability to replace cephalin in the prothrombin molecules, thus forming an inactive prothrombin which cannot be activated by calcium.

In previous papers we have shown the active coagulant of tissue extracts to be a protein-phospholipin compound consisting of about 13 phospholipin molecules (probably cephalin) united to one protein molecule. We have also shown that this coagulant enters directly into the formation of fibrin by a union between its phospholipin and blood fibrinogen through calcium atoms. The phospholipin of tissue fibrinogen, having only one point of union with the protein molecule, is very readily extractable—even by benzene at room temperature—but when a second point of attachment is obtained, as in fibrin, then this doubly-bound phospholipin becomes entirely non-extractable by lipoid solvents. We have presented analyses of fibrins containing various amounts of added tissue fibrinogen, showing that a part of the phospholipin is bound in a non-extractable form. We were led to the conclusion that the phospholipin molecules were just as essential in the clotting process as was the calcium, especially since removal of the phospholipin

from the tissue fibrinogen entirely prevented the latter from entering into the fibrin formation and instead changed it into an active anticoagulant.

On the basis of such a view of the clotting process as is outlined above it becomes a relatively simple matter to account for the anticoagulant action of the protein fraction of tissue fibrinogen. It is probably due entirely to its great phospholipin combining power by virtue of which it attaches to the free end of the cephalin molecules of the tissue fibrinogen, thus leaving no point for union to the blood fibrinogen through calcium.

Method of preparation of this tissue anticoagulant. Our original work in this direction was done with lung tissue in trying to discover whether both the protein and phospholipin fractions of tissue fibrinogen were essential for its coagulative activity. In order to prevent alteration in the protein during extraction of the phospholipin, we tried various lipoid solvents—absolute ether, alcohol, benzene, chloroform, carbon bisulphide and carbon tetrachloride,—finally arriving at the use of benzene as the one least harmful to the protein and the most readily recovered.

By thorough benzene extraction of dried and powdered lung tissue (calf and horse lungs were use mainly) at room temperature for several days, or until the benzene filtered off perfectly clear and colorless, we were able to transform the coagulant of such lung tissue into a strong anticoagulant. That is, whereas a saline extract of the dried lung originally acted as an extremely strong blood coagulant, a similar 0.9 per cent sodium chloride extract of the benzene extracted lung inhibited blood clotting. Such an anticoagulant extract may be made still more active by addition of small amounts of acid to precipitate any tissue fibrinogen molecules that still retain phospholipin. Thus, whereas the acid precipitate from an active coagulant extract of fresh calf lung contains 41.6 per cent phospholipin, the acid precipitate from one anticoagulant extract prepared contained only about 13 per cent phospholipin, and the anticoagulant globulin remaining in solution after the acid precipitation contained only 0.34 per cent phospholipin. This final globulin, almost phospholipin-free, was obtained by half saturating the solution with ammonium sulphate, since addition of acid sufficient to precipitate it seemed to greatly reduce its anticoagulative activity.

Since a globulin similar to the tissue fibrinogen of lung extract was found in extracts of various other tissues, we determined to see if this anticoagulant could be obtained from the other tissues as well. We chose liver for this purpose since it yields even a larger amount of

globulin than does lung. Liver and lung globulins are alike as regards precipitation reactions and appearance, but differ markedly in their action as blood coagulants, the liver globulin possessing very little such action as compared to that of the lung. We found, however, that we were able to prepare an even stronger anticoagulant from liver than from lung by benzene extraction, as above. This liver protein, when combined with lung cephalin, lost its inhibitory action and became as good a coagulant as we were able to obtain from replacing lung cephalin on the anticoagulant protein obtained from lung. Furthermore, the liver protein, when combined with cephalin prepared from the same liver tissue, gave just as good a coagulant as it did when combined with lung cephalin. This certainly indicates that liver globulin, as it naturally occurs, possesses only slight coagulative activity on account of the phospholipin it contains. This inactive phospholipin found here is probably identical with the "antiprothrombin" described by Howell. We hope soon to report more in detail a comparison of liver and lung phospholipin analyses in an attempt to account for their difference in regard to blood coagulation.

In a paper soon to be submitted, Dorst and Mills describe the preparation of anticoagulant proteins from lungs and livers of various kinds of animals, including mammals (horse and calf), birds (chicken) and reptiles (turtle). The method of preparation used was the same as above, merely thorough extraction of the dried tissue with benzene at room temperature. There seemed to be no specificity whatever in the anticoagulant action of the different preparations, each acting equally well to inhibit clotting of the blood of the different animals. In fact, the strongest preparation for any blood was obtained from turtle liver, that is, it inhibited clotting in horse plasma more than did a similar preparation from horse liver. This entire lack of specificity speaks strongly for our theory that each of these proteins acts as an anticoagulant only by virtue of its high cephalin-combining power, this preventing clotting in a manner mentioned on a previous page.

We should also mention here that it was found possible to destroy the anticoagulant action of the mammalian, bird or turtle preparations by adding cephalin prepared from any of these animals. Not only was such action lost, but the resulting compounds in every case were actually thromboplastic for the blood of each animal.

Chemical nature of the tissue anticoagulant. As has been mentioned before, the tissue fibrinogen (coagulant) and the protein fraction alone (anticoagulant) both exhibit the solubility characteristics of globulins.

They differ markedly however in their precipitation by acid, the former coming down with extreme ease and the latter with much greater difficulty. The anticoagulant alone gives a clear, transparent, somewhat brownish solution, readily filterable. The protein coagulates at about 56°C. The tissue fibrinogen solutions are always highly opaque, non-filterable and also coagulate at 56°C. By shaking cephalin with the anticoagulant solution it takes on exactly the appearance of the tissue fibrinogen solution.

Nitrogen determinations (Arnold-Gunning modification of the Kjeldahl method) on the two substances (made from calf lung) gave:

Tissue fibrinogen: (made by acid precipitation and washing free of albumins and salts)..... { 10.63 per cent N. / 10.76 per cent N.

Protein fraction: (after alcohol and ether extraction of 41.6 per cent phospholipin)..... { 16.01 per cent N. / 16.10 per cent N.

Anticoagulant globulin: (made from benzene extracted calf lung tissue, as described, and after alcohol and ether extraction of 0.34 per cent phospholipin after purification)..... 16.22 per cent N.

Phosphorus determinations (Pemberton-Neuman method) on these same samples gave:

Tissue fibrinogen:..... { 1.52 per cent P. / 1.56 per cent P.

Protein fraction: (described above)..... { 1.06 per cent P. / 1.06 per cent P.

Anticoagulant globulin: (described above)..... { 0.06 per cent P. / 0.01 per cent P.

Phospholipin: (extracted from tissue fibrinogen).... { 2.13 per cent P. / 2.12 per cent P.

Like analyses of these materials prepared from horse lung gave the following results:

Tissue fibrinogen..... { 10.67 per cent N. / 1.51 per cent P.

Protein fraction: (after extraction of 36 per cent phospholipin)..... { 16.00 per cent N. / 0.84 per cent P.

Anticoagulant globulin: (globulin purified by precipitation with ½ sat. $(NH_4)_2SO_4$. No acid precipitation)

a. One sample from lung after extraction of 1.2 per cent phospholipin)..... 0.12 per cent P.

b. Second sample from liver (after extraction of 4.6 per cent phospholipin)..... 0.11 per cent P.

Phospholipin fraction: (forming 36 per cent of tissue fibrinogen)..... 2.13 per cent P.
2.08 per cent P.

From these analyses there appears to be a great discrepancy in the amount of phosphorus present in the protein portion of tissue fibrinogen, depending on the method of preparation of the protein. Thus, by taking the whole coagulant molecule (from calf lung) and extracting its 41.6 per cent phospholipin by boiling alcohol and ether, a protein fraction is left which contains 1.06 per cent P., or with the horse lung preparation, after removing 36 per cent phospholipin, 0.84 per cent P. is left in the protein. As opposed to this, if we prepare the anticoagulant globulin from benzene extracted lung, and remove its small amount of phospholipin, we find 0.01 per cent to 0.12 per cent P. in the protein. Remembering that we take the entire globulin fraction of the tissue extracts in each case and that the nitrogen percentages check rather closely, it is unlikely that we are dealing with two different proteins, but rather that we are having a portion of the phosphorus of the phospholipin left with the protein in the alcohol and ether extraction in the first instance, while this phosphorus is mainly left in the water of the tissue extracts on precipitation of the globulins, or else is not split off from the phospholipin during benzene extraction of the dried lung in the second case. It is known that glycero-phosphoric acid does split off from the phospholipins with great readiness. We are investigating this further at present.

A very suggestive point in regard to the phosphorus of the coagulant is that, if we consider all the phosphorus to be in the 41.6 per cent phospholipin, then this phospholipin would contain 3.70 per cent phosphorus. Considering the phospholipin to be cephalin, since cephalin seems to be the only one of this class of compounds capable of aiding in blood clotting, and taking the molecular weight of the cephalin as about 835 (Thudicum (7) Koch (8)) it should contain 3.72 per cent phosphorus. Actual determinations of phosphorus in various samples of cephalin by different authors have given figures varying from 2.60 per cent to 4.42 per cent, but most of them are quite close to the above theoretical figure.

Our figures of 2.08 per cent to 2.13 per cent P. are for our total extracted material, and of course not on a purified phospholipin. That we have lost little or no phosphorus in the extraction is evident if one calculates the percentage phosphorus in the coagulant from the figures obtained for the protein and phospholipin fractions. Such calculation gives 1.52 per cent P. which agrees with one of our determinations on the whole substance.

So, although we have no proof that the phospholipin present in the coagulant is all cephalin, or that the phosphorus is present only as phospholipin phosphorus, the above calculations render this somewhat likely. Of course, lecithin might be present as well as cephalin, so far as phosphorus percentages are concerned, but lecithin is inactive as regards blood clotting, and we have shown (9) that the phospholipins of this compound are bound and rendered non-extractable when the compound takes part in fibrin formation in the proper porportions.

That any of the phosphorus is present as phospho-protein is rendered unlikely by the figures given for the anticoagulant globulin. One atom of phosphorus to a protein molecule would necessitate at least about 0.2 per cent P., while our findings varied from 0.01 per cent to 0.12 per cent P.

On the whole, it appears that the tissue fibrinogen is probably composed of a protein molecule containing no phosphorus in its make up and having a nitrogen content of about 16.2 per cent, to which protein molecule are attached about 13 molecules of a phospholipin, presumably cephalin. We should point out here that the compound may not always have the same relative amounts of protein and phospholipin in different species of animals, although we have found different preparations from the same species to check closely. Thus two preparations from calf lung showed 41.6 per cent phospholipin, while one from horse lung showed 36 per cent.

It is to be remembered that this compound possesses a further capacity for union with cephalin, as is evidenced by a fourfold increase in its coagulative activity on saturating it with cephalin. As its phospholipin is removed by benzene extraction it rapidly loses its coagulative action and soon becomes a strong blood anticoagulant.

Manner of action of tissue anticoagulant in inhibiting blood clotting. That our anticoagulant does inhibit blood clotting is evident from the data given below in table 1. It is here compared in about a 0.4 per cent solution with a 0.1 per cent solution of hirudin, and the neutralizing action of cephalin on each is also shown.

It possesses this inhibitory action also when injected intravenously as is evidenced by a continuous flow of blood from the puncture hole after removing the needle. The lack of coagulability of this blood is very similar to that of hemophilia patients, where a needle puncture wound often bleeds freely for several minutes. One rabbit, after having received 5 cc. of a strongly anticoagulant solution, was killed and the

TABLE 1

CITRATED HORSE PLASMA	1 PERCENT $CaCl_2$	OTHER ADDITION	1 PERCENT CEPHALIN EMULSION	COAGULATION TIME AT 40°C.
cc.	*cc.*		*cc.*	
1	0.3			2′ 00″
1	0.3		0.1	1′ 30″
1	0.3	0.1 cc. 0.1 per cent hirudin		9′ 30″
1	0.3	0.1 cc. 0.1 per cent hirudin	0.1	6′ 30″
1	0.3	0.1 cc. 0.1 per cent hirudin	0.2	4′ 00″
1	0.3	0.05 cc. 0.4 per cent tissue anticoagulant		4′ 00″
1	0.3	0.10 cc. 0.4 per cent tissue anticoagulant		7′ 15″
1	0.3	0.15 cc. 0.4 per cent tissue anticoagulant		15′
1	0.3	0.20 cc. 0.4 per cent tissue anticoagulant		No clot in 3 hours Clotted next day
1	0.3	0.05 cc. 0.4 per cent tissue anticoagulant		3′ 00″
1	0.3	0.05 cc. 0.4 per cent tissue anticoagulant	0.05	55″ 20 minutes later 45″

blood collected. There were no clots to be found in the vascular system, and the blood collected remained perfectly fluid as long as it was observed (several days in an ice box). We have tried it only on rabbits and guinea pigs thus far.

To show that the anticoagulant probably inhibits clotting by destroying the coagulative action of the small amount of tissue fibrinogen (thromboplastin) normally in the plasma, we tried mixing it with lung extract in varying proportions. The results are shown below in table 2.

It is evident, then, that this pure protein body, possessing a high cephalin-combining power, can neutralize the thromboplastic action of lung extract. As stated before, we presumed that this neutralization was brought about by a union of the anticoagulant protein molecules with the free point of attachment offered by the cephalin molecules of the tissue fibrinogen. This would effectually cover up the reactive group of the cephalin that normally functions in fibrin formation, and thus the tissue fibrinogen would be unable to unite through calcium to the blood fibrinogen. If such a union does occur between the anticoagulant and cephalin, then the cephalin, being bound at two points on its molecule instead of one, should become non-extractable by lipoid

TABLE 2

CITRATED HORSE PLASMA	1 PER CENT $CaCl_2$	LUNG EXTRACT (HORSE)	ANTICOAGULANT	COAGULATION TIME OF CONTROL	COAGULATION TIME AT 40°C. AFTER LETTING MIXTURES OF COAGULANT AND ANTICOAGULANT STAND AT ROOM TEMPERATURE FOR:					
					1 minute	5 minutes	10 minutes	30 minutes	1 hour	24 hours
cc.	*cc.*	*cc.*	*cc.*							
1	0.3			6′ 40″						
1	0.3	0.05		35″						
1	0.3		0.05	60′+						
1	0.3	0.05	0.05		45″	50″	50″	55″	60″	90″
1	0.3	0.05	0.15		1′ 30″					
1	0.3	0.05	0.75		2′ 30″					
1	0.3	0.05	0.75 H_2O	35″						

solvents, just as it does when combined in a similar manner in fibrin formation. To test out this theory the following experiments were performed:

SERIES A. *Solutions used:*

1. *Lung tissue fibrinogen* solution, containing 0.86 per cent coagulable material, and prepared from fresh horse lung by grinding up with 0.9 per cent NaCl solution, sedimenting and decanting, precipitating the globulin in the decanted solution by half saturation with $(NH_4)_2SO_4$, washing by resolution and reprecipitation, and finally redissolving in 0.9 per cent NaCl. A portion of this solution was boiled, the coagulum collected, washed free of salts and dried for analysis. Thirty-six per cent phospholipin was obtained by boiling alcohol and ether extraction.

2. *Lung anticoagulant* solution, containing 0.37 per cent coagulable material, was prepared from horse lung that had been dried at room temperature and thoroughly extracted with benzene at this temperature. The method of prepara-

tion and purification from here was the same as above. One and two-tenths per cent phospholipin was found in a portion of this material after heat coagulation, washing and drying.

3. *Liver anticoagulant* solution, containing 0.41 per cent coagulable material, was prepared from horse liver in a manner similar to the preparation of lung anticoagulant from the horse lung. Four and six-tenths per cent phospholipin was found in coagulum after washing and drying.

4. *Fresh egg white* solution, containing 1.38 per cent coagulable material, was made by dissolving 100 cc. of fresh egg white in 800 cc. of 0.9 per cent NaCl solution and filtering. A portion of this was boiled, the coagulum collected, washed and dried. Thirty-eight-hundredths per cent phospholipin was found in the dried material.

Experiment 1. One hundred cubic centimeters lung tisue fibrinogen solution were mixed with 400 cc. lung anticoagulant and the mixture kept for 8 hours at room temperature and 24 hours at 3°C. with frequent shaking by hand the greater part of the time. Tests as to the coagulative activity of the mixture were carried out as indicated in table 3. The mixture was finally boiled and the coagulum collected on a filter, washed free of salts and dried for analysis.

Phospholipin extractable = 7.5 per cent
Phospholipin calculated to be present = 14.1 per cent

Experiment 2. Eighty cubic centimeters lung tissue fibrinogen were mixed with 640 cc. liver anticoagulant, and the mixture treated as described in experiment 1.

Phospholipin extractable from dried coagulum = 7.4 per cent
Phospholipin calculated to be present = 11.2 per cent

Experiment 3. One hundred cubic centimeters lung tissue fibrinogen were mixed with 400 cc. liver anticoagulant, and the mixture treated as described in experiment 1.

Phospholipin extractable from dried coagulum = 8.4 per cent
Phospholipin calculated to be present = 15.4 per cent

Experiment 4. One hundred cubic centimeters lung tissue fibrinogen were mixed with 400 cc. egg white solution, and the mixture treated as described in experiment 1.

Phospholipin extractable from coagulum = 4.2 per cent
Phospholipin calculated to be present = 5.2 per cent

This series of experiments shows clearly that the anticoagulant from either liver or lung does unite with and render non-extractable a considerable portion of the phospholipin of the tissue fibrinogen when mixtures are made as here described. Thus in experiment 1, whereas 14.1 per cent phospholipin is calculated to be present, only 7.5 per cent or slightly over half, can be extracted with boiling alcohol and ether. Likewise in experiment 3, only 8.4 per cent phospholipin can be ex-

tracted although 15.4 per cent is calculated to be present. The possibility of the missing phospholipin being lost in washing the coagulum in each case is ruled out, because the analyses of the original solutions for phospholipins were made on the coagulums obtained by boiling and washing free from salt. There should roughly be the same loss of phospholipin in every case, if there occurs any loss at all.

That this attachment of the anticoagulant to the tissue fibrinogen phospholipin reduces the coagulative activity of the molecules is evident from the data given in table 3.

TABLE 3

CITRATED HORSE PLASMA	1 PER CENT $CaCl_2$	OTHER ADDITION	COAGULATION TIME AT 40°C.
cc.	cc.		
1	0.3		4′ 15″
1	0.3	0.05 cc. lung coagulant solution	20″
1	0.3	0.05 cc. lung anticoagulant solution	5′
1	0.3	0.1 cc. lung anticoagulant solution	7′
1	0.3	0.2 cc. lung anticoagulant solution	14′
1	0.3	1.0 cc. lung anticoagulant solution	34′
1	0.3	0.1 cc. liver anticoagulant solution	11′ 30″
1	0.3	1.0 cc. liver anticoagulant solution	22′
1	0.3	0.25 cc. of mixture made in exper. 1	33″
1	0.3	0.45 cc. of mixture made in exper. 2	32″
1	0.3	0.25 cc. of mixture made in exper. 3	26″
1	0.3	0.25 cc. of mixture made in exper. 4	22″
1	0.3	0.20 cc. egg white solution used	3′ 45″

In table 3 we see that in experiment 1 the activity of the coagulant was reduced so that 0.05 cc. of it, in the presence of 0.2 cc. of anticoagulant, could only induce clotting in 33 seconds, instead of in 20 seconds as it did when taken alone. This represents a diminution in activity to about one-fourth, as we have shown elsewhere in our work. Along with this diminution to one-fourth in activity we find by analysis that almost one-half of the phospholipin of the coagulant has been rendered non-extractable.

Experiments 2 and 3 show much the same thing, although it is not so striking in 2, when the ratio of coagulant to anticoagulant in volume was 1:8. The same loss of activity is evident, but the relative amount of phospholipin rendered non-extractable is smaller.

In experiment 4, on the contrary, 4.2 per cent phospholipin was extractable, while only 5.2 per cent was calculated to be present. Also, we see in table 3 that the egg white solution had a weaker effect in decreasing the activity of the coagulant solution.

This series of experiments brings out clearly that the anticoagulant globulin does render non-extractable a considerable portion of the phospholipin of the coagulant at the same time that it reduces its coagulative activity. We desired to know, however, whether other protein solutions, besides egg white, would fail to produce this same result when mixed with the coagulant and the mixture boiled. Not only that, but we wanted to know whether a protein phospholipin compound similar to the lung coagulant except in possessing little coagulative power would likewise have its phospholipin rendered non-extractable. For this purpose we used tissue fibrinogen from the liver which possessed only a very weak coagulative action. As was to be expected, we found that albumins prepared from liver and lung were almost inert as regards combining with the phospholipin of the lung coagulant and reducing its activity. We also found that an active anticoagulant had very little influence on the liver tissue fibrinogen solution, so far as rendering its phospholipin non-extractable, but that this little effect was enough to destroy its coagulative action.

SERIES B. *Solutions used:*

1. *Lung tissue fibrinogen*, as described previously. Contained 0.64 per cent coagulable material, of which 36 per cent was phospholipin.

2. *Liver tissue fibrinogen*, made in similar fashion. Contained 0.67 per cent coagulable material, of which 26.7 per cent was phospholipin.

3. *Lung anticoagulant*, made as described in series A. Contained 0.57 per cent coagulable material of which 1.3 per cent was phospholipin.

4. *Lung albumin solution*, made from the lung extract used above after removing the globulins by half saturation with $(NH_4)_2SO_4$. The precipitate from complete saturation with $(NH_4)_2SO_4$ was redissolved and reprecipitated and finally dissolved in water to give 0.25 per cent of coagulable material, of which 2 per cent was extractable phospholipin.

5. *Liver albumin solution*, made in the way just described for lung albumin, and containing 0.12 per cent coagulable material, of which 4.7 per cent was phospholipin.

Experiment 1. Sixty cubic centimeters of lung tissue fibrinogen solution were mixed with 240 cc. of lung albumin solution, and the mixture treated as described in series A, experiment 1.

Phospholipin extractable from coagulum = 14.8 per cent
Phospholipin calculated to be present = 15.1 per cent

Experiment 2. Sixty cubic centimeters of lung tissue fibrinogen solution were mixed with 240 cc. of liver albumin solution, and the mixture treated as described.

Phospholipin extractable from coagulum = 21.0 per cent
Phospholipin calculated to be present = 22.4 per cent

Experiment 3. Sixty cubic centimeters of liver tissue fibrinogen solution were mixed with 240 cc. of lung anticoagulant solution and the mixture treated as described.

Phospholipin extractable from coagulum = 6.6 per cent
Phospholipin calculated to be present = 7.3 per cent

Experiment 4. Sixty cubic centimeters of liver tissue fibrinogen solution were mixed with 240 cc. of lung albumin solution, and the mixture treated as described.

Phospholipin extractable from coagulum = 10.0 per cent
Phospholipn calculated to be present = 12.0 per cent

The experiments of series B show conclusively that albumins, which are inert as regards blood clotting, are also incapable of binding the phospholipin of tissue fibrinogen to any more than a very slight extent. This is in great contrast to the experiments of series A, where almost half of this phospholipin was rendered non-extractable through the avidity of the anticoagulant protein for phospholipin attachment.

With the liver coagulant, which possesses little coagulative activity and appears to contain only a small part of its phospholipin as cephalin, we see that the anticoagulant protein combines only slightly. This slight combination was sufficient to destroy the thromboplastic activity of the compound. We are now studying this phospholipin of the liver compound in an effort to determine how it differs from that in the lung coagulant, and whether it is identical with Howell's antiprothrombin.

Discussion of results. Our findings here still further strengthen our views of the manner in which tissue fibrinogen acts in blood clotting. Having determined that the union between the tissue and blood fibrinogens was by means of the phospholipin present and the formation of a calcium salt (fibrin), that the phospholipin in this union became in part bound in a non-extractable form in the fibrin, and that previous removal of the phospholipin from the tissue fibrinogen with benzene prevented the latter from entering into fibrin formation, the next step was to see if we could leave the phospholipin in place but destroy its effectiveness by satisfying its combining power before adding it to the blood plasma. To get a suitable substance with a high cephalin combining power, all that was needed was to extract the phospholipin from some tissue fibrinogen itself and use the protein fraction. This

protein, when mixed with other whole molecules of the coagulant should unite with the free point of ionization of the phospholipin and thus leave no point for union with blood fibrinogen through calcium. Our findings reported here show conclusively that such a union does occur between the anticoagulant protein and the tissue fibrinogen phospholipin, and also that the coagulative activity of the coagulant is thereby reduced.

Whether the anticoagulant of peptone plasma and hirudin act in a similar manner, we cannot yet say, although it is very likely since they, like our anticoagulant, lose their inhibitory power when treated with the proper amount of cephalin. It is possible that there are many other substances capable of thus uniting with the free bond of the tissue fibrinogen phospholipin and thus decreasing its activity. It may be that nucleic acid acts in this way instead of by decalcifying, although no one has ever shown nucleic acid to be capable of combining readily with phospholipins. So far we have experimented only with the anticoagulant globulin described in this paper.

SUMMARY OF RESULTS

1. Whereas normal lung tissue yields a globulin which is a very active blood coagulant, lung tissue dried at room temperature and thoroughly extracted with benzene at this same temperature yields a globulin possessing a very strong anticoagulant action.

2. This transformation of an active coagulant to an active anticoagulant involves only the thorough removal of the phospholipin from the coagulant, leaving a protein with a high phospholipin-combining power.

3. This anticoagulant globulin appears to contain no phosphorus in its protein molecule, the small amount found probably being an impurity from decomposition of the phospholipin with a splitting-off of glycerophosphoric acid.

4. The globulin fraction from various tissues was found to be capable of yielding this anticoagulant protein, there being no apparent specificity even for widely divergent species. The strongest anticoagulant for any blood was obtained from turtle liver.

5. Normal liver globulin, although only a very weak coagulant as compared to lung globulin, becomes a very strong anticoagulant upon extraction of its phospholipin.

6. The anticoagulant globulin is capable of uniting with the free bond of the tissue fibrinogen phospholipin and thus reducing its coagulative activity. Experiments, with phospholipin analyses, show this conclusively. As high as almost half the phospholipin of tissue fibrinogen was found to be thus united with and rendered non-extractable with lipoid solvents (boiling alcohol and ether).

7. Other proteins such as tissue albumins, lacking a high phospholipin-combining power, are unable to act in this way, so that practically the entire phospholipin content remains extractable.

8. Liver "tissue fibrinogen," possessing only a slight coagulative activity and presumably having only a small part of its phospholipin content as cephalin, reacts to only a small extent with the anticoagulant globulin.

9. The anticoagulant action of the globulin is readily destroyed and an active coagulant formed by the proper addition of cephalin.

10. The conclusion is justified that this protein owes its anti-coagulative action to its high cephalin-combining power.

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THE ACTION OF ULTRA-VIOLET RAYS ON STARFISH EGGS

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In living protoplasm, as in other physical systems, where radiant energy is absorbed, it produces physical and chemical effects; these may secondarily lead to changes of composition or structure within the system and thus alter its activity. In some cases these changes may be of such a kind that the reparative processes of living matter are unable to restore normal conditions, and permanent injury or destruction results; in other cases the effects produced may be temporary and similar to those of normal activating or stimulating agents. When substances of specific light-absorptive properties are present naturally, definite physiological effects frequently follow radiation with light of wave-length corresponding to the specific absorption of the substances; the chlorophyll-containing organs of plants and the various photoreceptors of animals illustrate this condition; an analogous sensitivity may be artificially imparted to many light-insensitive forms of protoplasm by the introduction of dyes or other compounds (case of eosin and other photodynamic substances). The more pronounced physiological effects produced in unspecialized forms of protoplasm by the shorter as compared with the longer wave-lengths of visible light and by the ultra-violet are probably to be correlated with the greater absorption of these rays. Probably a main reason for the relatively great efficiency of ultra-violet rays as cytolytic and activating agents is that their effect is concentrated in the protoplasmic surface-layer or plasma membrane, the region which is primarily altered in stimulation and cytolysis. In general the physiological effects produced by these rays do not appear to be qualitatively different from those of other physical and chemical agents which act upon this region of the cell.

The study of the general physiological action of radiation is perhaps best approached by investigating the action of ultra-violet rays on unspecialized forms of protoplasm. Most living cells are little affected

in their activity by the longer rays of visible light. As the wave-length shortens the physiological activity of the rays appears in general to increase; *e.g.*, the phototropic reactions of both animals and plants are shown especially in the blue and violet region of the spectrum. In the region just beyond the visible rays the general intensity of physiological action increases rapidly as the wave-length shortens. The irritant, stimulating or cytolytic effects of even brief exposure to rays of 2600 to 1800 Ångström units are well known. At present this knowledge is chiefly of a general and qualitative character. The more precise correlation of wave-length with physiological action in the ultra-violet region of the spectrum is a subject for future investigation.

For a comparison of the action of ultra-violet radiation upon the cell with that of other physical and chemical agents the starfish egg has many advantages. Its properties and behavior under normal and experimental conditions have been the subject of much investigation at Woods Hole and elsewhere; the eggs are abundant and easily obtained during the whole summer; and the unfertilized egg responds in a highly definite manner (membrane-separation, cleavage, activation) to treatment by a variety of physical and chemical agents.

The experiments described in the present paper were performed at the Marine Biological Laboratory, Woods Hole, during the summer of 1921. The source of ultra-violet light was a small mercury arc quartz lamp, with an arc about 8 cm. long, running on a potential of 100 to 110 volts with a direct current of 1½ to 2 amperes. The lamp was enclosed within a rectangular wooden box 12 inches square by 20 inches high, painted black inside and out, one side forming a hinged door, and provided with holes below and above for ventilation. In most of the following experiments the eggs were exposed in a layer of sea water 1 cm. deep in a flat-bottomed uncovered glass dish, placed inside the box vertically below the horizontal arc; they were allowed to settle before exposure to the rays, and never formed more than a single layer thick. The rise of temperature inside the box was only a few degrees while the lamp was running; during the longer exposures the temperature of the sea-water containing the eggs was kept approximately constant by surrounding the dish with cold water; the temperature was observed at the beginning and end of the radiation period; it lay usually between 18° and 20° and never rose sufficiently to produce direct effects on the eggs. In most experiments the distance of the bottom of the dish from the arc was 14 to 15 cm., and the quantity of radiation received by the eggs was varied by varying the time of exposure. It was found that constancy

of experimental conditions was more readily secured in this way than by varying the distance of the eggs from the arc.

Most of the following experiments were performed on unfertilized eggs. Ultra-violet rays produce in those eggs various definite effects of which the following were those chiefly studied: *1*, structural changes; *2*, direct activation effects; *3*, changes in the response of previously radiated eggs to sperm-fertilization and to artificial activation by heat and butyric acid. A partial study was also made of the action of the rays on recently fertilized eggs, and on the motility and fertilizing power of the spermatozoa.

It is well known that the general physiological state of starfish eggs and their responsiveness to fertilization and artificial activation vary according to the time which has elapsed since their removal from the body of the animal to sea-water. The eggs are immature and unfertilizable when removed; about 10 minutes later (at 20°), when the germinal vesicle begins to break down, they become at once completely responsive to both sperm-fertilization and artificial activation and preserve this responsiveness without apparent change until the separation of the first polar body; at this time there is a decline of responsiveness, and with the completion of maturation the susceptibility to fertilization undergoes still further decrease, although without disappearing entirely. In correspondence with these three periods, the experiments on unfertilized eggs were divided into three groups, in which the eggs were exposed to radiation, *1*, before the breakdown of the germinal vesicle; *2*, during the maturation period, *i.e.*, between the breakdown of the germinal vesicle and the separation of the first polar body; and *3*, in the early post-maturation period, between 1 and 3 hours after the separation of the second polar body.

ACTION OF ULTRA-VIOLET RAYS ON UNFERTILIZED EGGS. Under normal conditions the full-grown starfish egg remains immature, with its large germinal vesicle intact, until it is removed from the body cavity of the animal into oxygen-containing sea-water. Between 10 and 15 minutes later the nuclear membrane becomes indistinct and disappears, and in about 50 and 75 minutes, respectively (at 20°), the two polar bodies are separated. The physiological effects produced by activating agents vary in a highly characteristic manner with the state of maturation. If within 6 to 8 minutes after removal, before any visible change has occurred in the germinal vesicle, the eggs are exposed for 2 or 3 minutes to warm sea-water (30° to 35°) or fatty acid (*e.g.*, 0.005 n butyric acid in sea-water), the breakdown of the germinal vesicle is

completely prevented and the eggs remain permanently immature.[1] As Delage first showed,[2] the dissolution of the germinal vesicle is associated with a sudden and remarkable change in the physiological properties of of the egg, which then for the first time becomes fertilizable; it is only in the interval of about 30 to 35 minutes between this event and the separation of the first polar body that the egg is completely responsive to sperm-fertilization and to artificial activation by heat and butyric acid. With the separation of the first polar body the eggs lose much of their susceptibility to activation by these agents, and a further decline of susceptibility occurs at the second maturation division.[3] After maturation is complete the effects of sperm-fertilization are variable and irregular; although the mature eggs may be fertilized, usually only a small proportion develop normally. Fatty acid and warm sea-water used as activating agents in the post-maturation period induce only irregular cleavage followed by early breakdown.

It is remarkable that certain parthenogenetic treatments, *e.g.*, mechanical agitation[4] and treatment with unbalanced isotonic salt solutions (*e.g.*, pure NaCl solution[5]) are more effective after maturation is complete than before; these methods, however, never give rise to more than a small proportion of swimming larvae. The activating influence of ultra-violet rays, according to our observations of last summer, is also somewhat more pronounced during the post-maturation period.

Direct structural changes produced by ultra-violet radiation. A frequent effect of brief radiation (4 to 15 minutes) is local injury of the cell-surface, shown in the protrusion of small rounded vesicles of clear protoplasm; these are found chiefly in one hemisphere, presumably that exposed more directly to the rays, and usually do not reach their maximum development until 2 or 3 hours after radiation; apparently they indicate local interruption or breakdown of the plasma-membrane. The degree of these effects increases with the duration of radiation; more prolonged radiation of this intensity (*i.e.*, with the arc at 14 cm. from the eggs) leads to complete granular disintegration of the egg protoplasm. Eggs which have been radiated for 10 to 15 minutes

[1] R. S. Lillie: Journ. Exper. Zoöl., 1908, v, 375, *cf.* p. 400; Biol. Bull., 1917, xxxii, 131, *cf.* footnote p. 135.

[2] Delage: Arch. d. Zoöl. expér. et gén., Ser. 3, 1901, ix, 285.

[3] That this is true of activation by heat and spermatozoa is shown in the above cited paper in Journ. Exper. Zoöl., *cf.* pp. 396, 411; with fatty acid the conditions are the same as with heat.

[4] A. P. Mathews: This Journal, 1901, vi, 142.

[5] R. S. Lillie: This Journal, 1910, xxvi, 106; *cf.* p. 119.

often exhibit at the surface one or more local crescentic areas of broken-down granular protoplasm, sharply delimited within from the rest of the egg, which appears normal. These areas are evidently produced by local breakdown of the surface layer of protoplasm, followed by the formation of a new surface-film by the unaltered protoplasm beneath; they are frequent also in eggs which have been injured mechanically (*e.g.*, by microdissection)[6] or by local osmotic disruption resulting from temporary exposure to dilute sea-water.[7] They are interesting chiefly as illustrating the direct cytolytic action of the rays. The formation of new surface-films beneath such areas is an example of the normal structure-forming or reparative activity of the living protoplasm. Small local injuries in starfish eggs are often thus delimited; more extensive injuries, however, although at first confined to one hemisphere, frequently spread and involve the whole egg; thus eggs which have been radiated for an hour or more are usually found 2 or 3 hours later to be completely coagulated. Such a locally disintegrated area is always bound externally by a thin film which is often seen to be continuous with a partly separated membrane surrounding the whole egg and evidently of the same nature as the "fertilization-membrane" which separates on fertilization or artificial activation. In general we have found the susceptibility of starfish eggs to the cytolytic action of ultra-violet rays to be greatest in the post-maturation period.

Activation by ultra-violet radiation alone. In the starfish egg the activation effects caused by radiation alone are comparatively slight; complete activation like that induced by heat or fatty acid does not occur, at least under the conditions of our experiments. Membrane-separation, usually imperfect, is a frequent but not invariable result of brief radiation (2 to 7 minutes with arc at 14 cm.); this result is most readily obtained in the post-maturation period and is followed by slow irregular cleavage in many eggs; it is seldom, however, that development continues to the blastula stage, although a few such cases were observed. More prolonged radiation, 10 to 20 minutes or more, prevents the lifting of the fertilization-membrane from the egg-surface; and many eggs thus treated cleave without showing visible membranes.

One of the effects of the rays is apparently to alter the physical consistency of the fertilization-membrane and decrease its extensibility. Eggs which were radiated for 5 minutes or longer and then fertilized always showed imperfect separation of membranes; and in eggs which

[6] R. Chambers: This Journal, 1917, xliii, 1; *cf.* p. 6.

[7] Unpublished observations of my own.

were fertilized and radiated about 20 minutes later, after the membrane was visibly lifted from the surface, its further separation was hindered or prevented; afterwards such eggs showed the membrane more widely separated on one side than on the other.[8] The separation of fertilization-membranes by treatment with warm sea-water or butyric acid is also decreased or prevented if the eggs are exposed to the rays for a few minutes before the treatment. In order to cause evident interference with membrane-separation, more than 2 minutes' exposure to the above radiation is required, and the effect increases as the radiation is prolonged. In eggs radiated for 10 or more minutes and then fertilized, membranes do not separate but remain closely applied to the egg-surface.

Apparently the activating influence of the rays is accompanied by some other action unfavorable to development; this second action makes it impossible to induce complete parthenogenetic development by radiation alone. In normal eggs a radiation (at 14 cm.) of even 1 minute duration, applied either before or after fertilization, retards cleavage and interferes appreciably with development, and this injurious effect increases rapidly with the duration of radiation. It is remarkable, however, that in cases where the responsiveness to fertilization is less than the optimum, *e.g.*, in eggs naturally defective or in normal eggs fertilized during the post-maturation period, a brief radiation applied previously to fertilization often has a favorable influence on the subsequent development (see below, p. 65.) We have also found that the partial activation resulting from brief exposure to the rays may shorten considerably the time of exposure required for activation by heat and fatty acid. This effect is obtained, however, only with durations of radiation too brief to produce marked injury (*cf.* page 66).

Effects of radiation applied before breakdown of germinal vesicle. It has been mentioned that certain activating treatments (heat, butyric acid) inhibit the breakdown of the germinal vesicle if they are applied immediately after the removal of the eggs from the animal; and Clowes has

[8] This lack of symmetry in the action, together with the above-described cytolytic effects on one side of the egg, indicates a high degree of opacity to ultra-violet rays. The latter must therefore produce their effects chiefly or entirely by altering the protoplasmic surface-layer. Compare the results of Redfield and Bright on the Nereis egg (Journ. Physiol., 1921, lv, 61); the photographs of these authors indicate that in this egg ultra-violet rays (2800 Å.U.) are almost completely absorbed by the surface-layer. As in Asterias, local cytolytic effects and activation are produced by the rays.

recently shown that the same effect may be produced by spermatozoa.[9] We were unable, however, to prevent or retard this process by ultra-violet rays. This absence of inhibitory effect is in correspondence with the relatively slight activating properties of the rays. In three series of experiments eggs were radiated (at 14 cm.) for periods ranging from 1 to 60 minutes, exposures beginning between 3 and 8 minutes after removal. Brief exposures (1 to 4 minutes) produced no appreciable change in the eggs; while longer exposures had essentially the same effect as with eggs radiated during the early maturation period, *viz.*, partial activation followed by slow irregular cleavage in many eggs (*ca.* 50 per cent with 10 to 20 minutes' radiation). The only difference was that the activation effect was somewhat delayed, presumably until after the nuclear membrane had broken down.

Effects of radiation on the response to sperm fertilization. Eggs exposed to ultra-violet rays for varying periods, and then transferred to normal sea-water and fertilized with normal spermatozoa, show retarded and irregular cleavage and abnormal development, the degree of these effects varying with the intensity and duration of the radiation. As will shortly be described, however, an apparent exception to this rule may occur in the case of defective eggs or eggs radiated in the post-maturation period; in such cases brief radiation may have a favorable influence upon the subsequent development.

A large number of experiments (more than 30) were performed in which unfertilized eggs were radiated at different times before, during and after the maturation period and then fertilized. In each of these experiments the eggs from a single animal were used; these were exposed to the light from the arc (at 14 cm.) in the manner already described. At intervals portions were transferred to normal sea-water in a series of dishes; and at the close of the entire period of radiation a portion of each lot of radiated eggs and a control of unradiated eggs were fertilized at the same time. The subsequent behavior of the radiated eggs, both fertilized and unfertilized, was compared with that of unradiated eggs.

As already described, radiation of more than a few minutes' duration interferes with the separation of the fertilization-membrane. The rate and character of the succeeding cleavage are also readily altered; eggs that have been exposed to rays of the above intensity for 1 to 2 minutes show distinct retardation in the rate of cleavage and increase in the proportion of irregular cleavages; and the degree of these effects increases

[9] G. H. A. Clowes: Journ. Biol. Chem., 1920, xli, Proc. Amer. Soc. Biol. Chemists, p. li.

with increase in the time of exposure; in general an exposure of about 20 minutes is required for the complete prevention of cleavage in eggs radiated before the separation of the first polar body, while one of 15 minutes is sufficient in the post-maturation period. When cleavage is markedly retarded it never proceeds far, and the eggs break down without further development.

TABLE 1

NUMBER OF EXPERIMENT	TIME AT WHICH RADIATION WAS BEGUN	APPROXIMATE PERCENTAGE OF EGGS FORMING BLASTULAE AFTER RADIATION OF THE FOLLOWING DURATIONS						
		0 (Control)	1 minute	2 minutes	4 minutes	7 minutes	10 minutes	15 minutes
				Series A				
	minutes after removal							
1	32	90	90	90	40	25	0	0
2	3	90	90	90	90	10	10	0
3	30	90	90	90	85	80	35	1
4	3		95	90	70	65	50	1
5	36	90	90	90	50	35	1	0
6	30	90	90	85	25	Few	0	0
Average		90	90	90	60	35	15	<1
				Series B				
	hours after removal							
1	3	5	25	20	5	1	0	0
2	3	*ca.* 90	50	40	40	35	25	0
3	3	*ca.* 30	50	35	25	20	1	0
4	$2\frac{1}{4}$	*ca.* 70	?	60	1	1	1	0
5	3	50	50	30	2	1	1	0
6	2	75	40	20	20	5	0	0
7	3	25	25	10	5	1	<1	0
Average		50	45	30	14	9	4	0

The most convenient index of the impairment in developmental capacity is furnished by the proportion of eggs developing to a blastula stage. Table 1 gives the approximate percentage of eggs forming blastulae in two typical series, of six and seven experiments respectively, carried out in June, 1921. In series A the exposure to the rays began during the maturation period; in series B after maturation had been completed for some time.

It will be noted that in two of the six experiments of series A (nos. 2 and 4) radiation had begun before the germinal vesicle had broken down; the results of these experiments, however, did not differ significantly from the others. In series B the eggs had all been mature for some time.

The differences between the two series are characteristic. Eggs radiated for 1 or 2 minutes during the prematuration period show little or no decrease in the proportion reaching the blastula stage, although the blastulae are typically less active and show a greater number of abnormalities than the control. But with 4 minutes' radiation there is a distinct decline in developmental power, and few eggs radiated for 15 minutes form larvae. In series B the resistance to injury is distinctly less than in series A; there is a well-marked decline in developmental power after 2 minutes' radiation, and in only two out of the seven experiments does a significant proportion of eggs radiated for 7 minutes reach the blastula stage.

In other experiments performed later in the season (August), in which the effects of briefer exposures (1 to 5 minutes) on fertilization and developmental power were especially studied, a similar difference was observed. Eggs radiated for 4 and 5 minutes during the post-maturation period frequently yielded less than 1 per cent of blastulae. As already pointed out, even under normal conditions the post-maturation period is an unfavorable one for fertilization; a greater susceptibility to injury and less power of recovery also characterize this period of the egg's history. The fact that certain of the less effective parthenogenetic treatments, *e.g.*, mechanical agitation, pure NaCl solution and ultra-violet radiation, give their best results during this period is probably to be correlated with the increased alterability or lessened stability indicated by the above experiments.

In a considerable number of instances the curious and apparently paradoxical effect was observed that eggs which had been radiated for brief periods ($\frac{1}{2}$ to 2 minutes) shortly before fertilization yielded more numerous and more active larvae than untreated eggs fertilized at the same time (*e.g.*, expers. 1 and 3, series B). This effect was found only in those cases in which the eggs gave a poor response to fertilization, as is frequent in the post-maturation period. Apparently the condition rendering these eggs resistant to activation is in part removed by brief radiation. It has been known for some time that analogous effects may be produced by other agents; for example, treating naturally resistant starfish eggs, shortly before fertilization, with weak solutions

of ether or chloral hydrate or with pure isotonic NaCl solution may greatly increase the proportion of eggs developing to larval stages.[10] Apparently in this case the preliminary treatment results in a facilitation of the activation-process. There is definite indication that ether produces its effect on these refractory eggs by altering the state of the plasma membrane; and it seems not improbable, especially when other closely related facts are considered, that the essential general condition underlying such effects is a modification in the film-structure of the protoplasmic system, especially of its surface-layer. Thus it is known that anesthetization (*e.g.*, by alcohols) renders the eggs of starfish and sea-urchins resistant to certain forms of artificial activation, *e.g.*, by pure isotonic salt solution;[11] this interference almost certainly depends on a modification of the plasma membrane (the surface-layer of the entire egg), involving increased resistance to alteration by the salt-solution (*i.e.*, a "stabilization" effect). A change of properties of the reverse kind would presumably lessen the resistance to activation, and thus favor a more complete response to fertilization.

Influence of brief radiation on activation by high temperature. Brief exposure to ultra-violet rays also modifies in a characteristic manner the response of unfertilized eggs to artificial activation. Experiments of two kinds were performed with high temperature as the activating agent. In one series the eggs were radiated for a few minutes during the maturation period, and then treated with warm sea-water (32° to 34°) for the periods required for partial or complete activation; in the other series the two treatments were similar but their order was reversed.

Radiation followed by warm sea-water. Immediately after radiation the eggs were transferred to warm sea-water (kept at the experimental temperature in a beaker) and at intervals portions were transferred to normal sea-water. Ten experiments, each with eggs taken from a single animal, were performed with a previous radiation of 2 to 4 minutes' duration, and two with one of 6 minutes' duration. In nine out of the ten experiments of the first group the minimal duration of exposure to high temperature required to produce blastulae was decidedly shortened as a result of the previous radiation, and the percentage of blastulae resulting from a definite exposure of less than the optimum duration was increased. The optimum duration of exposure is also correspondingly shortened by preliminary radiation, but the blastulae resulting from optimum exposure are typically fewer and more abnormal with

[10] R. S. Lillie: Biol. Bull., 1911, xxii, p. 328.

[11] R. S. Lillie: Journ. Exper. Zoöl., 1914, xvi, 591.

radiated than with unradiated eggs, a result indicating that some degree of injury is associated with the partial activation produced by the rays. In the two experiments with 6 minutes' radiation and in one with 4 minutes' radiation the radiated eggs in all cases yielded fewer blastulae than the corresponding unradiated eggs.

The experiments summarized in table 2 are typical. The temperature of the warm sea water was 33°. The figures give the approximate percentages of eggs developing to a blastula stage. It is clear from these results that brief radiation shortens, approximately by 1 to 2 minutes, the time to produce parthenogenetic development by exposure to a temperature of 33°. In normal eggs about 6 minutes is the optimum for this temperature.[12]

TABLE 2

DURATION OF EXPOSURE TO 33°	EXPERIMENT 1 AUGUST 1, 1921, RADIATED FOR 2½ MINUTES		EXPERIMENT 2 AUGUST 1, RADIATED FOR 4 MINUTES		EXPERIMENT 3 AUGUST 2, RADIATED FOR 3 MINUTES	
	Unradiated	Radiated	Unradiated	Radiated	Unradiated	Radiated
minutes						
1	0	1 larva seen	0	0	0	0
2	0	*ca.* 1%	0	0	0	<1%
3	<1%	5–10%	0	0	0	*ca.* 1%
4	<1%	*ca.* 25%	1 larva seen	*ca.* 2%	*ca.* 10%	15–20%
5	5–10%	*ca.* 90%	*ca.* 1%	35–45%	20–25%	80–85%

Warm sea-water followed by radiation. Reversing the order of the two treatments appears to abolish, or at least greatly to decrease, the activation-furthering effect of brief radiation. No constant results were obtained in eleven experiments in which eggs which had been exposed to 33° for 3 to 6 minutes were immediately afterwards radiated (at normal temperature) for periods varying from 1 to 5 minutes. Radiation of 4 and 5 minutes in all cases decreased the number of larvae; in a few instances eggs radiated for 2 and 3 minutes produced blastulae with briefer exposures to 33° than unradiated eggs, but the difference was within the error of such experiments. Apparently partial activation by high temperature decreases the susceptibility to the activating influence of radiation; this suggests that the material or structure acted upon by ultra-violet rays is the same as that affected by high temperature.

[12] R. S. Lillie: Biol. Bull., 1915, xxviii, 260; *cf.* table vi, p. 276.

Influence of brief radiation on activation by butyric acid. The procedure in these experiments was similar to the above, with the difference that a solution of butyric acid (0.004 and 0.005 n) in sea-water was substituted for the warm sea-water. On the whole, the results obtained were closely similar to those with warm sea-water. In five out of six cases a preliminary radiation of 2 to 4 minutes caused a definite increase in the responsiveness of the eggs to the butyric acid; *i.e.*, exposures of a given duration (less than the optimum) produced a larger proportion of blastulae from radiated than from unradiated eggs. In three experiments with radiation lasting 5 and 6 minutes there was no increase of responsiveness and the resulting blastulae were more abnormal than those from unradiated eggs.

Five experiments with the reversed order of the two treatments gave no definite results.

ACTION OF RADIATION ON MOTILITY AND FERTILIZING POWER OF SPERMATOZOA. The motility of spermatozoa is very rapidly affected by ultra-violet rays; and the rate of injury, as shown by loss of fertilizing power, is much more rapid with these cells than with the eggs, probably because of the minute size of the elements and the relatively large proportion of surface-protoplasm. In three successive experiments in which moderately dilute suspensions of sperm from three different animals were exposed to the rays in shallow vessels covered by quartz plates, at distances from the arc of 9.5, 13.5 and 25 cm. respectively, motility had almost ceased after exposures of 2, 4 and 10 minutes. Even with the weakest intensity (25 cm.) motility was reduced by one minute's exposure to apparently less than half the normal.

Normal eggs fertilized with radiated sperm develop imperfectly, if at all, as shown by delayed and irregular cleavage and failure to reach advanced stages. Sperm radiated for one minute at 9.5 cm. proved still effective in enabling most eggs to form blastulae; but of eggs fertilized with sperm radiated for 2 minutes at 9.5 and 13.5 cm. less than 1 per cent formed blastulae; with radiation at 25 cm. and exposures of 4, 6 and 8 minutes the proportions of eggs forming blastulae were respectively 50 per cent, less than 1 per cent, and zero. The decline in fertilizing power is thus rapid, although apparently less so than the decline in motility.

Activation by radiated sperm shows the characteristic features of partial activation; *i.e.*, the effect is similar to that obtained by insufficient exposure to heat or butyric acid. It is noteworthy that sperm radiated for 10 and 15 minutes at 9.5 cm. still caused membrane-

separation in normal eggs, although without inducing cleavage. It is known, however, that even dead sperm may produce membranes under certain conditions; such sperm does not enter the egg.[13]

GENERAL CONCLUSIONS. In general the effects produced in unfertilized eggs by brief radiation resemble those of partial activation; and in fact a temporary exposure to high temperature (32°), too brief to cause membrane-formation, has precisely the same kind of effect; *i.e.*, subsequent treatment of such eggs with warm sea-water or buytric acid solution produces larvae with briefer exposures than in the case of normal eggs, and the optimum duration of exposure is decreased.[14] Brief treatment of normal eggs with butyric acid solution has a similar effect, the only difference being that with this agent fertilization-membranes are formed with briefer exposures than with warm sea-water.[15]

Effects of this kind seem to be related to those usually termed "sensitizations," in which without undergoing evident alteration the cell acquires increased susceptibility to the action of a stimulating, cytolytic or activating agent. In starfish eggs the effective duration of an activating treatment may also be shortened by simultaneous exposure to weak solutions of ethyl alcohol (3 to 4 vols. per cent) in sea-water, although no appreciable activation is produced by this solution acting alone.[16] There is an evident resemblance between this effect and the facilitation of sperm-activation by radiation and ether, described above. An incomplete activation, insufficient for the separation of a fertilization-membrane, may be experimentally indistinguishable from a sensitization.[17]

Apparently the immediate change produced in the egg-system by ultra-violet radiation is of the same essential nature as that caused by temporary exposure to high temperature or other activating agent.

[13] *Cf.* J. Loeb: Artificial parthenogenesis and fertilization, Univ. of Chicago Press, 1913, Chapter xix.

[14] *Cf.* p. 288 and table xvii, p. 292, of my paper on heat activation in Biol. Bull. 1915, xxviii, 260.

[15] *Loc. cit.*, tables xiv–xvi, pp, 289–291.

[16] See my paper in Journ. Biol. Chem., 1916, xxiv, 233, footnote p. 246, for a brief account of experiments on activation with butyric acid in presence of ethyl alcohol. Experiments with heat activation gave similar results: *e.g.*, the most effective exposure to 34° in normal sea-water, 2½ to 3 minutes, was shortened to 2 to 2½ minutes in sea-water *plus* 1 vol. per cent alcohol, and to *ca.* 1½ minutes in 1.5 per cent alcohol (unpublished observations of June, 1915).

[17] Compare the sensitization to hypertonic sea-water produced in Arbacia eggs by exposure to isotonic solutions of NaCl and similar salts: R. S. Lillie and M. L. Baskervill: This Journal, 1921, lvii, 110.

In the case of heat-activation, the high temperature coefficient of the process indicates that the immediate change produced in the protoplasmic system is of a structural rather than a purely chemical kind, and consists in a change in the physical state of the colloidal substratum.[18] Taken as a whole, the evidence supports the view that in both normal and artificial activation a temporary structural change (possibly in the nature of a de-emulsification or breakdown of film-structure) occurs in the surface-layer ("cortical zone") or plasma-membrane of the egg; and that this change secondarily—possibly by removing barriers to diffusion or by changing the electrical polarization of partitions —permits the chemical interaction of specific substances which previously were kept apart.[19] This critical reaction in some manner initiates or "releases" the sequence of metabolic and structural changes determining development.

Tchahotine has recently shown that an appreciable structural alteration involving increase of permeability is produced in sea-urchin eggs by localized ultra-violet radiation.[20] It has been known for some time that ultra-violet rays cause membrane-formation and partial activation in Arbacia eggs,[21] a fact which we have confirmed; and the conditions are probably not essentially different in the Asterias egg. Hence the conclusion seems justified that the primary effect of the rays in the starfish is a structural change—*i.e.*, a change in the colloidal substratum of the protoplasm and probably of the surface-layer—upon which follow specific chemical effects as a secondary consequence. This con-

[18] R. S. Lillie: Biol. Bull., 1915, loc. cit.; *cf.* pp. 294 *seq.*

[19] *Cf.* the recent book of F. R. Lillie, Problems of fertilization, Univ. of Chicago Press, 1919, Chapters 5 and 6, for evidence that specific chemical unions form an essential part of the fertilization reaction. This evidence is strengthened by the recent observation that traces of Cu salts inhibit the immediate response of the egg to fertilization and artificial activation (F. R. Lillie: Biol. Bull. 1921, xli, 125).

There is evidence from other fields of investigation that specific chemical combinations frequently result from alteration or breakdown of protoplasmic structure. The acceleration of autolysis by cytolytic agents is apparently an example of this effect. An interesting case is the photogenic reaction in luminescent organisms, *i.e.*, the union of luciferin and luciferase; all classes of cytolytic agents produce this reaction which, as Harvey has recently shown, gives evidence of specific character (E. N. Harvey: Journ. Gen. Physiol., 1922, iv, 285; for the action of cytolytic agents *cf.* the same author's paper in Biol. Bull., 1921, xli, 280).

[20] S. Tchahotine: Compt. rend. Soc. de Biol., 1921, lxxxiv, 464. Compare the results of Redfield and Bright on the Nereis egg (*loc. cit.*).

[21] J. Loeb: Science, 1914, xl, 680.

clusion again emphasizes the analogy between activation in unfertilized eggs and stimulation in irritable protoplasmic systems in general.[22] The physiological action of radiation cannot be understood without taking into account the similar effects produced by other agents.

SUMMARY

1. Brief exposure (2 to 7 minutes) of unfertilized starfish eggs to the rays from a quartz mercury lamp (arc *ca.* 8 cm. long with 1½ to 2 amperes current, distance 14 cm. from eggs) causes imperfect separation of fertilization-membranes followed by slow cleavage. The activation produced is only partial, although occasionally a blastula is formed. Complete activation is never obtained with radiation alone, apparently because of the injurious secondary action of the rays. Longer radiation causes local cytolytic effects which may spread and involve the whole egg.

2. Normal eggs exposed to ultra-violet radiation for varying times and then fertilized with normal sperm exhibit abnormalities of the following kind, which increase with the duration of radiation: interference with separation of fertilization-membranes; retarded and asymmetric cleavage; irregular development and early cessation of development. The susceptibility to injury and also to activation by rays is greatest in the post-maturation period.

3. In the case of eggs which give an imperfect or partial response to sperm-fertilization, a brief radiation applied shortly before fertilization often has a favorable influence upon the subsequent development.

4. Brief preliminary radiation of unfertilized eggs shortens the time required to produce activation by fatty acid and high temperature. Radiation following partial activation by fatty acid or heat is ineffective in promoting activation or injurious.

5. Ultra-violet rays rapidly decrease the motility and destroy the fertilizing power of spermatozoa.

[22] The changes in the "cortical zone" of egg cells during activation are apparently of the same essential nature as those occurring in the plasma membranes of irritable cells during stimulation (*cf.* my discussion in Journ. Exper. Zoöl., 1914, xvi, 591, pp. 611 *seq.*).

THE ACTION OF ULTRAVIOLET LIGHT ON EGG ALBUMIN IN RELATION TO THE ISOELECTRIC POINT

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Ultraviolet light of wavelength less than 300 or 330 $\mu\mu$ produces marked physiological effects. Light filtered through glass, containing only wavelengths greater than 330 $\mu\mu$, has no effect except in the presence of sensitizers. A number of observers have reported that one of the results of ultraviolet radiation is the coagulation of egg albumin (1), (2), (3). Burge (4) found that lens protein coagulates less easily than egg albumin. Pincussen (5), in reviewing the subject of light, says that colloids generally tend toward greater aggregation under ultraviolet light.

In a recent review (6) I have proposed a general theory of light action which may be summarized as follows. It is known that absorption of ultraviolet light, of wavelength less than 300 $\mu\mu$, usually results in the emission of electrons, so that a negatively charged substance loses its charge and an uncharged substance acquires a positive charge. This phenomenon is called the photo-electric effect. The number and velocity of these emitted electrons increase with decreasing wavelength of the exciting light (7), (8). For each substance there is a long wavelength limit to the effect, or a photo-electric threshold, so that all wavelengths shorter and no wavelengths longer than this threshold wavelength stimulate the emission of electrons. The threshold is fixed for every substance under given conditions, but is very subject to shifts with changes in surface conditions. The photo-electric effect is especially marked in metals, where ultraviolet light is absorbed in a surface film, but it is found in most solids and also in a great many liquids. In liquids, however, little light is absorbed in a surface layer so that few electrons are lost from the surface and the photo-electric effect becomes latent, the emission of electrons by atoms in the interior being usually shown by an increase in conductivity.

I have supposed that the first step in all the actions of light on organic substances is an emission of electrons from the substance affected, that is, the ionization of photo-electric molecules. This state of ionization may be likened to the latent image in a photographic plate and subsequent development of this latent image by chemical action leads to the various physiological light effects. In other words, the physiological actions of light are due to the formation of photochemical compounds, and the formation of these compounds is due to the photo-electric action of light which, by producing an ionized condition in light sensitive molecules, leads to subsequent chemical reactions. The photodynamic sensitization of substances, so that they react toward visible light, in the presence of certain dyes, in the same way that the untreated substance acts toward ultraviolet light, is explained thus. In the presence of a sensitizer the photo-electric threshold of the light-sensitive substance is shifted toward longer wavelengths, so that it is now photo-electric at these wavelengths and is therefore ionized, with resultant chemical action, by visible light.

To make this theory tenable it must first be proved that the effect of ultraviolet light is due to the emission of electrons from light-sensitive atoms and molecules. Secondly, it must be proved that this emission takes place in visible, as well as in ultraviolet light on the addition of a sensitizer. The second part of the proof is still wanting, but the results of this paper, on the coagulation of egg albumin by ultraviolet light, furnish excellent evidence for the first part.

Suppose, as a premise, that the absorption of ultraviolet light by the particles of colloidal egg albumin results in the emission of electrons. These colloidal particles are known to have no charge at the isoelectric point (pH 4.8), a negative charge on the alkaline of the isoelectric point (pH greater than 4.8), and a positive charge on the acid side (pH less than 4.8). On radiating with ultraviolet light less than 330 $\mu\mu$ one would therefore expect the following results. In solutions with pH greater than 4.8 the particles should lose their negative charge, and some would acquire a positive charge, precipitating the others as coagulated albumin. In these solutions radiation would lead to greater aggregation. For pH = 4.8, the particles being uncharged would, on radiation, acquire a positive charge and repel each other. This would give greater dispersion. For pH less than 4.8 the particles have a positive charge to begin with and, on radiation, this charge would become greater, giving greater dispersion.

If radiated through glass there should be no change in the solutions, unless a sensitizer is present, since egg albumin is not photo-electric for wavelengths greater than 330 $\mu\mu$. That egg albumin is photo-electric for wavelengths less than 330$\mu\mu$ has been shown by Schanz (9) and has also been confirmed in this laboratory.

The loss of electrons on the absorption of ultraviolet light should also affect the action of colloids toward electrolytes. The precipitation of colloids by electrolytes is supposed to be due to neutralization or reversal of the electric charge on the colloid particle by the opposite charge on the precipitating ion. In the particular case of precipitation of egg albumin by ammonium sulphate we may picture the action of the electrolyte in the following way. As soon as the pH of a solution of egg albumin is made less than 4.8 some of the positively charged colloidal particles are precipitated by half saturation with ammonium sulphate. This is due to the bivalent anion SO_4'', which is known to be an effective precipitating agent toward positively charged colloids. The smaller the pH of the solution, the greater the positive charge on the particles of egg albumin and the greater the precipitation with half saturation, which becomes practically complete at pH 3.0. The experiments were not carried below pH 3.0. For pH greater than 4.8 there is no precipitation at half saturation but it occurs when a greater amount of ammonium sulphate is added. Now Sorensen (10) has shown that in a solution of egg albumin, containing ammonium sulphate, the albumin combines with the ammonia ion on the alkaline side of the isoelectric point. The amount of $(NH_4)^+$ combined with the negatively charged albumin particles increases with increasing pH and also with the concentration of ammonium sulphate. It therefore seems probable that this combination with $(NH_4)^+$ reverses the sign on the colloidal particles when the concentration of ammonium sulphate is great enough. They then become positive and can be precipitated by the SO_4'' ion, so that, although half saturation with ammonium sulphate does not precipitate negatively charged egg albumin, a greater concentration will do so.

After radiation, the solution with pH 4.0, having a greater positive charge should give a larger precipitate with half saturation than before radiation, and solutions with pH 4.8, 5.6, 6.2, 6.8, 7.2 and 7.6, which give no precipitate with half saturation before radiation, might be expected to give at least a partial precipitation after radiation.

These results, predicted theoretically on the assumption that the action of ultraviolet light on egg albumin is photo-electric, are entirely confirmed by the results given below.

METHOD. The white of a fresh egg was diluted to twice its volume with distilled water. The globulins were precipitated by half saturation with ammonium sulphate and removed by centrifuging and filtering. The albumin solution was then dialysed for two days against distilled water which was frequently changed. The solution thus obtained was found to have a pH of 6.2. By means of indicators, solutions were prepared with pH ranging from 4.0 to 8.2. They were exposed to the quartz mercury arc, in quartz and glass test tubes, for a succession of fifteen-minute periods, at a distance of five inches. The temperature of the solutions did not rise above 32°C. during the exposure.

RESULTS: I. *Effect of radiation through glass.* No effect was found at any pH when a solution was exposed to the quartz mercury arc in a glass test tube.

II. *Effect of radiation through quartz.* If solutions with pH 4.0, 4.8, 5.6, 6.2, 6.8, 7.2, 7.6, 8.2 are radiated in quartz test tubes the following results are obtained. Solutions with pH 4.0, 4.8, 7.2, 7.6, 8.2 remain clear. Those with pH 5.6, 6.2, 6.8 become cloudy after an exposure of about twenty minutes. This cloudiness increases with length of radiation although in 6.8 it never becomes very great. In 6.2 the solution becomes very thick and milky but no precipitate forms and it can not be filtered clear. On half saturation with ammonium sulphate both 6.2 and 6.8 precipitate leaving a clear filtrate, which after long enough radiation is free from albumin. Before radiation no precipitate was formed with half saturation. In 5.6 a precipitate settles out spontaneously on radiating and the filtrate is clear, no ammonium sulphate being necessary to remove the albumin. In pH 4.0 more albumin is precipitated with half saturation after radiation than before. Solutions with 4.8, 7.2, 7.6 and 8.2, in spite of being clear after radiation, precipitate on half saturation with ammonium sulphate, whereas before radiation no precipitate was formed. In 4.8 a short radiation converts all the uncharged to positively charged albumin which is completely precipitated by half saturation. In 7.2, 7.6 and 8.2 a very long radiation is necessary to complete the change, the particles having a large negative charge to begin with. The fact that solutions with pH greater than 6.8 do not show opalescence after radiation is probably due to the fact that, at that distance from the isoelectric point, the initial dispersion of the solution is so great that visible aggregation does not take place even when some of the particles become positively charged. A 5.4 solution precipitates in fine particles, 5.8 in large loose flakes, and 6.2, though opalescent, does not precipitate at all, showing that, although

radiation gives a greater aggregation in all solutions with pH greater than 4.8, the degree of aggregation becomes steadily less with increasing alkalinity. A series of radiated tubes is shown in figure 1 and the results are tabulated in table 1.

The fact that it takes longer to complete the reaction in 6.2 than in 7.2 is probably due to the heavy opalescence in 6.2 which decreases the penetration of the light.

III. *The nature of the precipitate.* Schanz (3) has published work showing that, after radiation with ultraviolet light, egg albumin is more easily precipitated by half saturation with ammonium sulphate. He took this to mean that the albumin had been changed to globulin.

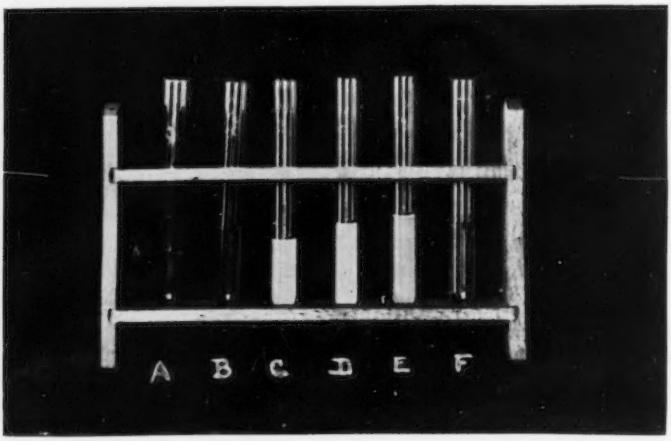

Fig. 1. A series of tubes containing egg albumin radiated one hour in quartz tubes. Tube *A* has pH = 3.8; tube *B*, 4.8; tube *C*, 5.6; tube *D*, 6.2; tube *E*, 6.8; tube *F*, 7.8. The difference in the opacity of *C*, *D* and *E*, which was in reality very marked, does not show in the plate.

This, however, is the wrong interpretation. The precipitate that settles out on radiating a pH 5.6 solution is not globulin but coagulated albumin for it does not dissolve in 1 per cent sodium chloride or in concentrated hydrochloric acid. The albumin is changed by radiation and becomes positively charged albumin, after which it is precipitated like globulin by half saturation with ammonium sulphate.

IV. *Change in hydrogen ion concentration of radiated solutions.* Since the hydrogen ion concentration of a solution has an effect on the nature and amount of the electric charge on the colloidal particles, it is possible

that the discharge of electrons from the colloidal particles might influence the hydrogen ion concentration of a solution. Observations were impossible, by the colorimetric method used, in the region pH 4.8 to 6.8 where the solutions became opalescent on radiation. Solutions with pH 3.6 and 7.2 were therefore radiated in quartz and glass test tubes and compared every hour with unradiated controls. In neither case was the solution radiated through glass affected even after a four-hour exposure. The pH 3.6 solution remained unchanged after three hours' radiation through quartz but showed a slight change in the

TABLE 1

Effect of ultraviolet radiation on egg albumin (through quartz)

pH	CHARACTER OF SOLUTION AFTER RADIATION	EFFECT OF ONE-HALF SATURATION WITH $(NH_4)_2SO_4$ BEFORE RADIATION	EFFECT OF ONE-HALF SATURATION WITH $(NH_4)_2SO_4$ AFTER RADIATION	TIME OF RADIATION NECESSARY TO COMPLETE THE PRECIPITATION BY ONE-HALF SATURATION
				hours
4.0	Clear	Small precipitate	Heavy precipitate	1¼
4.8	Clear	No precipitate	Heavy precipitate	1¼
5.6	Precipitate in heavy flakes. Clear on filtering	No precipitate	Heavy precipitate	1¼
6.2	Thick opalescence on filtering	No precipitate	Heavy precipitate	3¾
7.2	Clear	No precipitate	Heavy precipitate	3
7.6	Clear	No precipitate	Heavy precipitate	4
8.2	Clear	No precipitate	Heavy precipitate	Not complete in 4 hours

direction pH 3.4 after four hours. The pH 7.2 solution however began to show a change at the end of an hour and after four hours radiation through quartz it had a pH of 6.8. The change was permanent since a radiated solution left over night, before the indicator was added, also had a pH of 6.8.

V. *The effect of sensitizers.* In the presence of dyes the action of light on egg ablumin through quartz may be delayed or hurried, and in the presence of some the same reaction takes place through glass that ordinarily takes place only though quartz. The pH of the egg albumin solution was 6.2 before the dyes were added. In adding the dyes,

0.1 cc. of a 0.1 N aqueous solution was added to 1.0 cc. of the solution of egg albumin (table 2).

When eosin, erythrosin or rose bengal is added to a solution of egg albumin at pH = 4.8 a precipitate is formed on radiation which is not formed in the unsensitized solution at this pH. This may be due to a shift toward greater pH on the addition of the sensitizer, or else the isoelectric point of egg albumin may be different in the presence of a dye. Either explanation is likely and this point will be tested by means of electrometric hydrogen ion determinations.

TABLE 2

Sensitizers

SUBSTANCE	PRECIPITATED BY ONE-HALF SATURATION	PRECIPITATED BY RADIATION AND ONE-HALF SATURATION		TIME OF RADIATION NECESSARY FOR COMPLETE PRECIPITATION WITH ONE-HALF SATURATION	
		Quartz	Glass	Quartz	Glass
				hours	*hours*
Albumin + eosin	−	+	+	1⅓	4
Albumin + erythrosin	−	+	+	1⅔	3⅓
Albumin + rose bengal	−	+	+	2⅓	Not complete in 2⅓ hours
Albumin + methylene blue	−	+	+	2⅓	Not complete in 2⅓ hours
Albumin + uranin	−	+	+	Not complete in 2⅔ hours	Not complete in 2⅔ hours

Non-sensitizers

				hrs.	
Albumin + fuchsin	−	+	−	2⅔	
Albumin + methyl orange	−	+	−	3	

Since the coagulation of egg albumin by ultraviolet light through quartz is explained so perfectly on the assumption that it is due to photo-electric action, it seems more than probable that the coagulation through glass, in the presence of a sensitizer, is due to the same cause. This would mean that the photo-electric threshold of egg albumin is shifted to longer wavelengths in the presence of a sensitizer.

CONCLUSIONS

It has been shown that, due to the photo-electric action of ultraviolet light on egg albumin, radiation produces a state of greater aggregation

when the albumin particles are negatively charged and a state of greater dispersion when they are uncharged or charged positively.

Although other colloidal solutions have not been worked with as yet, the principle is undoubtedly of general application and should explain, in a simple and satisfactory way, many of the physiological actions of light. So far no attention has been paid to the importance of the nature of the electrical charge on a substance that is radiated by ultraviolet light. A study of the effect of light on body tissues from this point of view promises most interesting results.

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THE PHYSIOLOGICAL RÔLE OF THE INTESTINAL MUCOSAL MOVEMENTS

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The mucosa of the small intestine is usually looked upon as a secreting and absorbing mechanism. The villi, vascularity, lymphatics and glands make up an arrangement well adapted to such a function. In discussions on intestinal absorption mention is usually made of the fact that the villi manifest active movements, and the interpretation made that these movements probably play some part either in regulating the blood stream or in promoting the flow of chyle from the mucosa into the deeper lymph channels. The fact that the mucosa as a whole manifests rhythmical movements is seldom mentioned, neither is there any clear interpretation of the physiological significance of these movements.

The work forming the basis of this report was done for the purpose of obtaining data upon which might be built a physiological interpretation of the movements of the villi singly, and of the mucosa as a whole.

A number of earlier workers have made observations which bear upon the question. Exner (1) made a study of the motor activities of the intestinal mucosa relative to the effect of sharp foreign bodies. He observed that mechanical stimulation of the intestinal mucosa calls forth a sharp local retraction of the mucosa and a marked anemia. He introduced into the gastro-intestinal tract a large number of needles, some with the eyes turned forward, and some the reverse. After a certain length of time he killed the animals and made observation mainly relative to two points, the position and direction of the needles, and the extent of injury to the mucosa. He found that the needles passed freely along the tract, that the eyes of the majority of them were directed forward, and that there was no evidence of mucosal injury except in a very few cases. He concluded that the motor reaction of the intestinal mucosa is the main factor involved in the protective mechanism against sharp foreign bodies. A number of years later Mueller (2) repeated these experiments and obtained the same results. He also studied the effects

of sectioning the vagi and splanchnics upon the distribution of needles in the gastro-intestinal tract. He found the needles directed as in normal animals, mucosal injury just as rare, but that longer time was required for them to pass along a given distance. He agreed with Exner that the mucosal motor activities serve as a protective mechanism against sharp foreign bodies, and also concluded that the mechanism is inherent in the intestinal wall.

Gruby and Delafond (3) observed the villi in the living animal, and saw lengthening, shortening and lateral movements. They interpreted these movements to be connected with the regulation of the blood flow through the mucosa.

Magnus (4) in connection with his extensive work on the activities of intestinal muscle attempted to record the movements of the mucosal musculature. He failed to record any clear-cut movements. He concluded that if any movements occur they must be entirely local in character.

The work of Bruecke (5), Heidenhain (6) and Spee (7) would indicate that the movements of the villi contribute mainly to the flow of lymph from the intestinal mucosa.

Gunn and Underhill (8) succeeded in recording rhythmical movements of the mucosal musculature of the cat's intestine. Their work was done with isolated strips, but they concluded that these movements occur in the intact animal, and are probably concerned with the regulation of blood flow through the mucosa. They reasoned by analogy. They found the mechanism to be similar to that which controls the movements of the spleen, and based upon the hypothesis that splenic contractions serve as an aid in driving the blood through that organ, they reasoned that the movements of the intestinal mucosa serve the same purpose.

This brief résumé of the previous work in this field indicates that the usual views expressed at the present time relative to the rôle of intestinal mucosal movements are merely hypotheses based upon observations of the movements. So far as we know, none of the observers in the field have made any extensive studies on lymph and blood flow or secretory activity in connection with their observation and registration of the movements of the villi and mucosa as a whole.

In the present work we have attempted to test these hypotheses by combining observations on lymph flow, blood flow, secretory activity and absorption with those on mucosal movement. Dogs were used throughout. The mucosal fields were prepared and observed as described in a previous communication (9).

Relation of blood flow to villi and mucosal activity. Direct observation of an active field of the mucosa shows that the contractions of the villi tend to squeeze out the blood from the capillaries. This can be observed in a spontaneously moving field, but best in case a single villus is picked out and stimulated mechanically.

If the mechanism is primarily adapted and adjusted to regulate the blood flow, then it would be natural to seek for the primary stimulus in local circulatory changes. If for instance the movement is an emptying act, then one would expect a filling of the capillaries to set off the movement. If on the other hand it is a filling act, then a diminution in the amount of blood ought to set off the movements.

The following observations and experiments when interpreted as a whole may serve to throw some light on the question:

1. In a series of 80 dogs in which villi and mucosal movements were observed, notes were taken on the general condition of the animal, blood pressure, color of the tissues, the presence or absence of food, and also how long before the experiment the last feeding had been done. By noting these conditions one can rather accurately predict the activity and reactivity of the mucosa. A clear pink mucosa associated with a fairly normal blood pressure is as a rule very reactive, and usually shows considerable spontaneous activity which persists. A blue or pale mucosa is never good for a sharp or sustained activity, although it may show spurts of great activity. The mucosa of recently fed animals shows greater activity and reactivity than that of starved animals, and as a rule also shows greater vascularity.

2. Ligation of the mesenteric veins leading from the field of observation leads to vigorous activity of both the villi and the mucosa for a few minutes, then to a gradual diminution and cessation. If the ligature is removed when the diminution begins to set in, recovery is usually complete; but if the ligature is allowed to remain until movement has ceased, one seldom sees any sign of recovery.

3. Ligation of the mesenteric arteries leading to the segment under observation leads to the same general results as ligation of the veins; first as an increase in activity, then a diminution. The field of course in this case becomes pale instead of blue, as in the case of vein ligation.

4. Moderate changes in blood pressure in case the pressure is fairly normal to begin with, make no perceptible difference in the activity or reactivity of the field under observation. An abrupt fall of pressure of considerable magnitude is followed by an increased activity, then by a diminution. If the fall is great enough to eliminate the circulation

from the mucosa the picture is identical with that of ligation of the mesenteric artery. We have never observed any change in activity or reactivity in case of an abrupt rise in pressure above the normal level.

5. Asphyxiation of the animal leads to an augmentation of villi and mucosal activity.

6. The injection of N/50 acid in saline and also 2.5 per cent sodium bicarbonate augments the activity of the mucosa, the alkali being more active than the acid.

These observations show that the motor activities of the intestinal mucosa affect the local circulation, but would seem to indicate that circulatory changes in themselves from a purely mechanical standpoint play very little if any part in setting off the motor mechanism. Chemical stimulation is much more strongly suggested.

Relation of lymph flow to mucosal motor activity. Relative to lymph flow two viewpoints are possible; the villi movements may be considered as emptying movements or as sucking movements. They might also play the double rôle. In the first case the movements would serve to empty the central lacteal, and one would then naturally look for distention of the central lacteal to serve as a setting off stimulus. In the second case a decrease in pressure within the central lacteal should lead to an increase in movement. If they play the double rôle it is difficult to see how it would be possible for them to rest at any time.

On opening the intestine it often happens that the central lacteals are plainly visible, especially marked after a fat meal. Extended observations of fields of this type show clearly that the retraction of the villus does not completely empty it, nor is the size or appearance of the central lacteal appreciably altered. The interpretation in this case would be that the movement of the villus has nothing to do with the emptying of the central lacteal, or that the emptying is not complete. Mueller (2) reports having been able to see evidence of changes in shape and volume during activity. The results of our own observations on this point are somewhat indefinite, but on the whole we have not been able to corroborate his findings. In order to keep the lacteal clearly under observation it is necessary to use low magnification, not more than 25 diameters. An eye piece micrometer is of little value because the villus in its retraction executes a somewhat swaying movement and consequently changes its position relative to the rulings on the micrometer. If a higher magnification is used the slightest movement throws the villus out of focus, rendering accurate measurement utterly impossible.

Artificial distention of the lymph sinuses and central lacteal is a difficult matter. The presence of valves in the mesenteric channels, as well as valve-like structure at the base of the central lacteal, prevents any marked distention by inserting a cannula or a needle into the mesenteric channels and exerting pressure. This was done in a number of experiments with indefinite results. On the whole there was possibly a slight increase in activity.

Bearing on this point, however, is the repeated observation that villi movement is more often seen in a fed than in a starved dog. It is in these fed animals that the central lacteal is more commonly plainly visible; also the villi are plumper. It is rather easy to distinguish between a villus with a swollen epithelial covering and the plump fat villus of an absorbing gut. In the former case the villi look like a gelatin finger, the epithelium somewhat ridged and very shiny.

Another point worthy of note is that in case the central lacteal was plainly visible when the mucosa was first exposed, it remained visible for the time of the experiment which in some cases extended over an hour. In these cases the mesenteric lacteals became empty and were scarcely visible in a very short time after opening the gut. This observation indicates clearly that the central lacteal is not completely emptied by the contraction of the villus. Coupling this fact with the fact of a valve-like structure at the base of the central lacteal, and with the fact that if the mucosa is free from absorbable material the central lacteal remains comparatively full, it is suggested that the movement of the villus may may play the rôle of a suction pump.

A series of experiments was carried out in which a cannula was inserted into the thoracic duct, and the cannula then attached to a syringe. In this case the same objections hold as were mentioned above and, in addition, apparently considerable resistance is offered by the mesenteric lymph glands. In this way it was possible, however, to either increase or decrease the intralymphatic pressure. The effects of increasing the pressure were not sharp or marked, but were rather constantly in the direction of increased activity. By this device it was possible to greatly decrease the intralymphatic pressure, in fact, almost to empty the lymphatics. When this was done there was a momentary increase in villi activity, followed by a decrease.

In order to obviate some of these difficulties segments of the intestine in fed animals were tied off and all the lymphatics ligated. After a short time all the lymphatics became plainly distended. Fields for observation were then prepared in both ligated and unligated portions of

the gut, and the villi movements were compared. During the observations the ligated channels were opened and the lymph allowed to escape. It is not practical to first prepare a field for observation, then ligate the lacteals, for the reason that it is necessary to have a clean field for observational purposes; and on the other hand if there is no absorbable material in contact with the villi the mesenteric lacteals soon collapse and become visible with difficulty. If the observation is made, then the field covered with absorbable material, it is necessary before another observation is made to clean the field. Any form of washing or scraping sets the villi into activity, consequently the results are of little value. Attempts were made to color the central lacteal by feeding the animal fats stained with Sudan III and also with Scharlach R, but in not a single case was there the slightest evidence of the stain having found its way into the central lacteal. It was hoped that by this method the central lacteal would be made more visible, and thus better observations could be made relative to the question of changes in shape and size. The dye invariably passed out by way of the blood stream. The results of the dye experiments on the whole were negative.

The villi activity in the fields connected with ligated lacteals was more pronounced than in the control unligated fields. When during the observation the lacteals were cut and the lymph allowed to escape, the villi activity gradually diminished.

The introduction intravenously of a large volume of isotonic saline or dextrose increases the flow of lymph from the thoracic duct. A series of experiments was carried out to determine the effect of this procedure on the villi activity, and also to note any possible correlation between the rate of flow and activity. We found the intravenous administration of salt or dextrose to be one of the most effective means of arousing both villi and mucosal activity. We also noted as a constant fact that the flow of lymph persisted for a longer period than the increased villi activity. As little as 50 cc. of fluid was sufficient to call forth a pronounced reaction. The introduction of larger amounts, 300 cc. to 500 cc., not only caused a great increase in lymph flow and villi activity, but produced also a pronounced swelling of the villi. This swelling was due mainly to an increased amount of fluid in the epithelium, the size of the central lacteal was not perceptibly changed.

An attempt was made to keep the fluid injected within the vascular system by adding enough gum acacia to make the viscosity of the fluid approximately the same as that of the blood. We hoped by this procedure to throw some light on the question of vascular and lymphatic

distention as factors in setting off mucosal activity. We could note no difference in the rate of lymph flow or in villi activity, from that obtained from plain saline, with this exception—that the gum appeared toxic and the animals passed rapidly into a state of shock after which both lymph flow and villi activity rapidly diminished. The fluid appeared to pass from the vascular system practically as rapidly as normal saline or dextrose. This corroborates the experience reported by Bayliss (10). We were not able to obtain the special gum which he recommends as being much more satisfactory.

During the course of these experiments another observation was frequently made which is worthy of note. In case a loop of intestine is much handled, the mesenteric lacteals rapidly empty themselves. That this is merely local is indicated by the fact that an adjoining segment which has not been handled remains normal in appearance. This fact emphasizes the possible importance of the contraction of the intestinal musculature as a factor in promoting the flow of lymph.

Relation of villi activity to the rate of absorption. In a previous paper (9) it was reported that the activity and reactivity of the villi is greater in the upper than in the lower part of the intestine. This raises the question as to whether the rate of absorption holds the same relation, and also as to whether any parallelism which may exist can be accounted for on the basis of villi activity.

The comparative rate of absorption was studied at various levels of the small intestine, using sodium chloride, dextrose and sodium stearate. The animals were anesthetized with ether, a median longitudinal abdominal incision made and the small intestine pulled up. This was divided into four loops corresponding to duodenum, upper jejunum, lower jejunum and ileum. In case food material was present the intestine was first washed out and drained. For the sodium chloride experiments isotonic dextrose was used as a flusher, and for the dextrose experiments isotonic sodium chloride was used. In the soap experiments salt was also used for washing. The loops were carefully tied off, care being taken to get them as near the same size as possible. Into each loop was then placed a definite volume of material of known concentration. This was allowed to remain thirty minutes, the animal in the meantime being kept in the best condition possible. At the end of this time the remaining material was as completely removed as possible, the volume determined, and also the dextrose, salt or soap content. It was not possible to measure the volume of the soap removed because the water had been almost completely absorbed leaving only a thick sticky mass which had

to be scraped and flushed from the mucosa. The sodium chloride was determined by the Volhard-Harvey method. The dextrose was determined by the Lewis and Benedict method, and also by a modification of the Bertrand method. Instead of filtration through asbestos the determinations were carried out in centrifuge tubes and the cuprous oxide separated by centrifugation. The sodium stearate was acidified with sulphuric acid then shaken in a separatory funnel with chloroform, washed with distilled water, dissolved in alcohol, then titrated with N/10 alcoholic potassium hydroxide.

The following table is a summary of averages obtained from these experiments.

The figures presented in the table are based upon six dextrose, five sodium chloride, and six soap experiments. There was some variation between the different animals in that we were able to get better absorption in some than in others, but the contour of the absorption curve for the different animals was remarkably uniform and corresponded closely to the curve plotted on the basis of averages. Several points stand out rather clearly. The duodenum is a poor dextrose absorbing organ. In two of the dextrose experiments almost all the sugar was recovered and more fluid was removed than had been introduced. The dextrose was most rapidly absorbed in the upper half of jejunum. Absorption of dextrose in the ileum is about the same as in the duodenum.

The results with the sodium chloride in the duodenum were similar to those with dextrose. For the remainder of the intestine the absorption was progressively better lower down, the greatest increase being between the duodenum and the upper jejunum. These results are in close agreement with those recently obtained by Cobet (11).

It is not possible on the basis of these results to strike any parallelism between the rate of absorption of dextrose, sodium chloride or water and the comparative activity and reactivity of the villi.

Owing to the fact that the greater part of fat absorbed from the intestinal tract leaves by way of the lymph stream, it was thought that there might be a more suggestive parallelism between the rate of fat absorption and villi activity than in the case of dextrose and sodium chloride. This hypothesis was tested out by the procedure already described.

Care was taken to eliminate the liver and pancreatic secretions from the loops under observation. A ligature was placed on the duodenum below the entrance of the pancreatic duct, and the gut then flushed out with warm isotonic saline.

TABLE 1

Showing the relative rate of absorption of dextrose, sodium chloride and sodium stearate in the different parts of the small intestine

SEGMENT OF INTESTINE	INJECTED						RECOVERED						ABSORBED					
	Salt solution		Dextrose		Sodium stearate		Salt solution		Dextrose		Sodium stearate		Salt solution		Dextrose		Sodium stearate	
	cc.	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*
Duodenum	20.0	0.174	20.0	0.942	20.0	0.533	17.1	0.129	19.3	0.477	0*	0.304	2.9	0.045	0.7	0.465	20.0†	0.229
Upper jejunum	20.0	0.174	20.0	0.942	20.0	0.533	14.0	0.093	14.4	0.354	0*	0.256	6.0	0.081	5.5	0.588	20.0†	0.277
Lower jejunum	20.0	0.174	20.0	0.942	20.0	0.533	13.0	0.083	17.5	0.475	0*	0.261	7.0	0.091	2.5	0.467	20.0†	0.272
Ileum	20.0	0.174	20.0	0.942	20.0	0.533	12.5	0.082	18.6	0.480	0*	0.306	7.5	0.092	1.4	0.462	20.0†	0.227

* Not measured.

† Practically all fluid absorbed.

The sodium stearate was slightly alkaline to phenolphthalein. There was no evidence of injury to the mucosa. On the whole the absorption of the stearate was not so good as that of the dextrose or the sodium chloride, but enough to be measured.

The curve of fat absorption is seen to be similar to that for dextrose. One point of dissimilarity seems worthy of note. Water absorption in all the loops in the soap experiments was practically complete. The residue had to be scraped, brushed and flushed from the mucosa.

The results of the absorption experiments just tabulated speak clearly against mucosal motor activity as being the determining factor of the rate of absorption of sodium chloride, dextrose and sodium stearate from the small intestine.

Mucosal movements and intestinal secretion. Pavlov (12), in his discussion on the secretion of the succus entericus, points out that mechanical stimulation of the intestinal mucosa calls forth a secretion of watery juice practically free from enzymes. The introduction of pancreatic juice into the gut, and also the injection of secretin into the circulation, causes the secretion of a juice rich in enterokinase. He furthermore reports that the gut secretes progressively less from the duodenum down, practically none appearing in the ileum. These observations suggested to us that there might be some correlation between mucosal movement and secretory activity. Work is in progress on the question of the relation of the so-called secretin preparations to mucosal activity and intestinal secretion.

We have constantly observed that vigorous activity of the individual villi or of the mucosa as a whole is accompanied or closely followed by the appearance of a thick mucous coating over the mucosa, and also that the mucus appears much more abundantly in the upper than in the lower portion of the small intestine. The mucous secretion is especially marked after the administration of pilocarpine and nicotine. It also appears after repeated splanchnic stimulation, mechanical stimulation, and the application of local irritants. After nicotine the mucus is often mixed with chyle, indicating that the pressure within the villi has become great enough to force out through the epithelium at least a part of the contents of the central lacteal. This phenomenon is always associated with vigorous movement of the mucosa as a whole. We have never observed it in connection with isolated villi movement. In many instances it was clear that the central lacteal had actually ruptured, in others not. At this point it is worthy of note that Spee found an increase in size of the central lacteal in case of vigorous villi contraction.

It was also our experience that after the mucosa had become covered with mucus it was never possible to elicit a normal motor reaction. That this was not due to mere mechanical interference with movement by the mucus is indicated by the fact that its removal by scraping or flushing did not result in recovery of normal reactivity. We could with considerable accuracy predict our subsequent success or failure to induce mucosal movement by the amount of mucus on the surface of the mucosa when first exposed.

Two hypotheses suggest themselves in explanation of the above outlined observations. Either a single stimulus or several stimuli occurring synchronously set off mucosal movement and excite the secretion of mucus, or the mucus is forced or squeezed out by the movements, particularly of the mucosa as a whole. This does not necessarily mean that mucus never appears independent of movement. It does appear under such conditions, but much more slowly.

We find here the suggestion of a differentiation of function between individual villi movements and movements of the mucosa as a whole. This suggestion is based upon two observations, namely, that one frequently observes active individual villi movement with no evident deeper mucosal movement and with little or no mucus appearing; and again, that deeper mucosal movement with or without rhythmical villi movements is always followed by the appearance of mucus. This would lead one to give credence to Exner's interpretation that the motor reaction of the intestinal mucosa to foreign bodies is protective. But this is probably not the whole story. Exner's conclusions were drawn after observing the effect of placing needles in the intestinal tract—an extreme case of mechanical stimulation. It would be rather rare that the food mass in the intestine would be of such composition as to threaten mechanical injury to the mucosa. We have demonstrated repeatedly that milder mechanical stimulation, such as stroking the mucosa with a smooth glass rod, or directing a stream of warm dextrose or salt solution across the surface, calls forth a mucosal retraction. The same occurs when parasites move across the field under observation. We may then imagine that the food mass under normal conditions does the same thing. In case of the presence of sharp bodies the reaction would be more vigorous. The end result would be the throwing out of a mucous juice which would greatly facilitate the onward passage of the mass, and at the same time serve as a protective. The protective rôle is emphasized still more in the case of the presence of irritants.

The suggestion of a differentiation in function between the movements of the intestinal mucosa as a whole and of the individual villi recalls the often-seen statement that the movements of the outer gut wall contribute to the outflow of lymph. It seems highly probable that the contractions of the individual villi contribute to the flow of lymph from the central lacteal. The vigorous contractions of the mucosa do not do this, in fact they retard the flow from the central lacteal, but tend to force the lymph out into the channels more closely connected with the outer musculature. The contractions of the outer musculature then force it onward, a return being impossible because of the valve at the base of the central lacteal.

Relation of mucosal motor activity to the mixing and propulsion of the food mass. The motor reactions of the individual villi and the mucosa as a whole are not progressive but local in character. Consequently they cannot contribute directly to the forward movement of the food mass. On *a priori* grounds it is reasonable to assume that the movements of both the mucosa as a whole and of the individual villi serve as a local mucosal stirring apparatus, not only mixing the food with the juice from the intestinal glands, but facilitating absorption by keeping the mucosal cells in constant contact with a more or less constant concentration of absorbable products, leaving aside for the moment any possible rôle played by the lymph and blood stream relative to the carrying away of the absorbed products. We are inclined to ascribe this mixing and stirring rôle to the movements of the individual villi in particular.

SUMMARY AND CONCLUSIONS

1. By direct observation it can be seen that the contraction of the individual villi and of the intestinal mucosa as a whole forces the blood out of the local capillaries. However, neither mere distention or emptying of the villi capillaries alone constitutes the stimulus which sets off the movement. This is witnessed by the result of the experiment in which the mesenteric veins and mesenteric arteries connected with the segments under observation were ligated. Consequently one cannot interpret the mechanism as being primarily adapted to the regulation of the local circulation through mechanical stimulation.

2. The evidence presented indicates that mucosal movements, particularly those of the individual villi, are connected with the lymph flow. This conclusion is based mainly upon the facts that an increase in intralymphatic pressure results in an increased villi activity, and also that

the intravenous administration of lymphagogues increases their activity. This does not negate or even minimize the possibility of the activity of the outer musculature of the gut as being a factor of importance. The evidence indicates that vigorous contraction of the mucosa as a whole retards the outflow of lymph, inasmuch as in those cases lymph is forced out onto the mucosal surface, and in some instances the central lacteal ruptured.

3. No direct parallelism or connection can be demonstrated between the rate of absorption of soap, dextrose, sodium chloride or water, and villi activity, at least it is not the main determining factor.

4. A close connection between mucosal movements and secretion of mucus is pointed out. It is suggested that these movements may play an important part in the secretion of succus entericus. Thus they become a factor of importance in the passage of food along the intestinal tract, and possibly also in the activation of the pancreatic protease.

5. It is pointed out that the villi movements may play the rôle of a local stirring and mixing apparatus.

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PSEUDO-PARADOXICAL PUPIL-DILATATION FOLLOWING LESIONS OF THE AFFERENT PATHS

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In studying paradoxical pupil-dilatation induced by lesions of the afferent paths it was found that the affected pupil exhibited at times a form of dilatation quite independent of the paradoxical phenomenon. As this dilatation appeared mainly, though not exclusively, during the pre- and post-paradoxical period, the name pseudo-paradoxical seemed to fit it. The phenomenon was so striking that it seemed to warrant investigation on its own account. For the general method of study the reader is referred to previous papers (1), (2).

After section of one or more divisions of the fifth cranial nerve. In cat 1 the middle division of the right fifth cranial nerve was divided just outside the infra-orbital foramen. On the 5th, 7th, 10th, 13th, 14th, 28th and 31st days the right was the larger pupil (pseudo-paradoxical dilatation). On the 7th day adrenalin failed to elicit true paradoxical effects in the right eye but elicited them in that eye on the 10th, 13th and 28th days. On the 10th day the true paradoxical effects were most pronounced whereas pseudo-paradoxical dilatation on this day was less in evidence. On the 31st day the skin of the right side of the face in the vicinity of the operative wound was extremely hyperalgesic when tested for pin pricking at 3.0 grams pressure although this stimulus was readily tolerated on the corresponding portion of the left side of the face. On the 63rd and 66th days adrenalin failed to elicit true paradoxical effects in the right eye although pseudo-paradoxical dilatation was present before and during the early stages of ether and absent during deep anesthesia when, with the wink reflex absent, the right pupil was smaller than the left (2.5:4.0 mm.). On the 66th day the nerve was exposed and crushed moderately with an artery clamp. On the 69th, 72nd and 75th day the right side of the face in the vicinity of the operative wound was markedly hyperalgesic to pin-pricking and on these days pseudo-paradoxical dilatation was present in the right eye. On the

76th day pseudo-paradoxical dilatation though present in the right eye was less pronounced than on the previous days and on this day adrenalin elicited true paradoxical effects in the right eye. On the 77th day acute catarrhal disease of the upper respiratory passages developed and the animal was rejected as unfit for further study. In two other animals similar operations yielded similar results. In one of these animals on the 14th day after section of the middle division of the fifth cranial nerve on the right side well-marked pseudo-paradoxical dilatation was present in the right eye and adrenalin elicited a trace of true paradoxical dilatation in the same eye. On this day section of the right cervical sympathetic and of the left vago-sympathetic reduced but did not altogether abolish the pseudo-paradoxical dilatation in the right eye. On the 15th day the right vagus nerve was divided in the neck and this also failed to completely abolish the pseudo-paradoxical dilatation in the right eye.

After crushing the posterior divisions of the upper cervical nerves. In cat 2 the posterior divisions of nerves C ii, iii and iv were exposed and crushed with an artery clamp a little distance outside the spinal canal. Pseudo-paradoxical dilatation was observed in the right eye just after the operation and on the 7th, 10th, 14th, 17th, 21st, 22nd and 25th days. During the early stages of ether anesthesia the dilatation persisted and often became enhanced but it usually disappeared with the wink reflex. Occasionally as on the 25th day it persisted during deep anesthesia. Adrenalin elicited true paradoxical effects in the right eye on the 10th, and 17th days but failed to elicit them on 7th, 14th, 21st, 22nd and 25th days. On the 10th, 14th and 17th days the pseudo-paradoxical dilatation was less marked than on the other days. On the 25th day the spinal cord was transected at roots C iv. As artificial respiration had to be resorted to, the animal was considered unfit for further study.

In two other animals similar operations yielded similar results. All the animals were tested for hyperalgesia to pin-pricking but no definite hyperalgesia could be established in these upper cervical cases, the skin on both sides being apparently tough and insensitive.

After section of the muscles about the shoulder. In cat 3 the muscles about the left shoulder were cut as in the operation for exposure of the brachial plexus. Pseudo-paradoxical dilatation was observed in the left eye just after the operation as well as on the 3rd, 4th, 18th and 25th days. On the 8th day pseudo-paradoxical dilatation was absent in the left eye in which adrenalin elicited well-marked paradoxical effects on this day and a trace of paradoxical effects on the 25th day. On the

3rd, 4th and 18th days adrenalin failed to elicit true paradoxical effects and on the 28th day both pseudo- and true paradoxical phenomena were absent in the left eye. On this day the muscles about the left shoulder were re-cut. Pseudo-paradoxical dilatation was observed in the left eye immediately after this operation and on the 32nd, 35th, 39th, 40th and 42nd days. On the 35th day the pseudo-paradoxical dilatation though present was slight and on this day as well as on the 39th and 40th days adrenalin evoked true paradoxical effects in the left eye. On the 32nd and 42nd days adrenalin failed to elicit true paradoxical effects in the left eye.

After section of the muscles about both shoulder joints and crushing of one brachial plexus. In cat 4 the muscles about both shoulder joints were cut as in the operation for exposure of the brachial plexus. The nerve trunks of the right brachial plexus were then crushed with an artery clamp, the left brachial plexus being left untouched. Just after the operation the right pupil was a trace larger than the left. A few hours later the left was the larger pupil by 2.0 mm. and remained so until the 5th day when the difference between the pupils was not so great although the left still remained larger than the right (6.0:7.0 mm.). With occasional exceptions such as the 25th day the right was the larger pupil (pseudo-paradoxical dilatation) from the 10th to the 43rd day. From the 8th to the 39th day adrenalin evoked true paradoxical effects in the right eye. It was noted that when these were well marked the pseudo-paradoxical dilatation was either altogether absent as on the 25th day or considerably diminished. From the 3rd to the 8th day adrenalin failed to evoke unequivocal paradoxical effects in either eye. Pin-pricking tests at measured strengths showed a relative cutaneous hyperalgesia on the left fore limb and shoulder from the 1st to the 4th day. From the 4th to the 9th day the skin over both fore legs and shoulders was insensitive to pricking at 27.2 grams pressure. On the 10th and 11th days coincident with the appearance of pseudo-paradoxical dilatation in the right pupil cutaneous hyperalgesia was present in the right fore leg and shoulder. This hyperalgesia was not constant. It varied much in intensity from the 17th to 35th day. On some days, e.g., the 18th, 21st and 35th days sensibility for pricking at 27.2 grams pressure seemed to be nil on both sides. On these days it was observed that pseudo-paradoxical dilatation, though present in the right eye, was not as well marked as on occasions when cutaneous hyperalgesia was present in the right fore leg and shoulder. After the 35th day the cutaneous hyperalgesia persisted in the right fore leg and shoulder

and with this was constantly associated well-marked pseudo-paradoxical dilatation in the right pupil. On the 39th day adrenalin evoked a mere trace of true paradoxical dilatation in the right pupil. After the 39th day adrenalin failed to elicit true paradoxical effects in the right eye. On the 39th day the animal began to use the right fore leg in walking. On the 43rd day the right brachial plexus was exposed and again crushed. On the 45th and 46th days adrenalin failed to evoke true paradoxical effects in the right eye and pricking tests showed cutaneous hyperalgesia in the right fore leg and shoulder. On these days well-marked pseudo-paradoxical dilatation was present in the right eye (5.0:4.0 mm.). On the 47th day it was found that the operative wound was slightly infected. Under suitable treatment the wound healed in a few days. From the 47th to 67th day both pseudo-paradoxical and true paradoxical phenomena were absent in the right eye. From the 73rd to the 86th day pseudo-paradoxical dilatation was present in the right eye and adrenalin evoked a moderate degree of true paradoxical dilatation. From the 73rd day onward pricking tests showed persistent cutaneous hyperalgesia in the right fore leg and shoulder. On the 79th day the animal began to use the right fore leg in walking. On the 88th day pseudo-paradoxical dilatation was well marked in the right eye and persisted throughout all stages of ether anesthesia but was more marked in the lighter than in the deeper stages. Under light anesthesia it was found that pricking either fore leg caused wider dilatation in the right than the left pupil. On this day after adrenalin had failed to elicit true paradoxical effects in the right eye the cord was transected just below roots C v as verified at autopsy. After this operation the pseudo-paradoxical dilatation was somewhat reduced though still present in the right eye. The right and left vago-sympathetics were then cut. After this the pupils were of equal size and remained so.

In two other animals after section or crushing of one brachial plexus similar results were obtained. In cat 5 after injection of a 60 per cent solution of alcohol into the left brachial plexus pseudo-paradoxical dilatation was present in the left eye from the 29th to 91st day and coincident with it a well-marked cutaneous hyperalgesia was present in the left fore limb and shoulder. In cat 6 on the 19th day after injection of a 60 per cent solution of alcohol into the left brachial plexus pseudo-paradoxical dilatation was present in the left eye and adrenalin failed to elicit true paradoxical effects. From the 19th to the 27th day very cold weather prevailed and on the 28th day it was found that adrenalin evoked true paradoxical effects in the left eye from which the pseudo-paradoxical dilatation had completely disappeared.

After injury of one sciatic nerve. In cat 7 the left sciatic nerve was exposed and crushed with an artery clamp. On the 3rd day pricking tests showed cutaneous hyperalgesia on the upper portion of the left thigh. Coincident with this the right pupil exhibited well-marked pseudo-paradoxical dilatation. On this day the right and left vago-sympathetics were transected and two hours later the right pupil was larger than the left (2.5:1.5 mm.) On the 4th day adrenalin failed to elicit true paradoxical effects in the right pupil which still continued to exhibit pseudo-paradoxical dilatation (5.0:4.0 mm.).

Fig. 1. Pseudo-paradoxical dilatation in the right pupil on the 38th day after crushing the left sciatic nerve and 30 minutes after spinal cord transection between roots C iv and v.

In cat 8 after crushing the left sciatic nerve pseudo-paradoxical dilatation was observed in the right pupil from the 4th to the 38th day with the exception of a period extending from the 8th to the 14th day when the pseudo-paradoxical dilatation was scarcely appreciable or was altogether absent. The skin of the whole of the left leg from the thigh to the foot was hyperalgesic to measured pin-pricking during the whole of the period in which the right pupil exhibited pseudo-paradoxical dilatation and during this period also adrenalin failed to elicit true paradoxical effects in the right eye. During the period in which the right pupil failed to exhibit pseudo-paradoxical dilatation the skin of the left leg below the knee was insensitive to pricking at 27.2 grams pressure although a slight degree of cutaneous hyperalgesia persisted in the upper portion of the right thigh. During this period, i.e., from the 9th to the 14th day, the right pupil was generally more sensitive to adrenalin than its fellow. Pseudo-paradoxical dilatation when present

Fig. 2. Same animal as in figure 1, 10 minutes after cord transection between roots L iv and v. Pseudo-paradoxical dilatation abolished in the right pupil.

in the right eye was enhanced by excitement and by pin-pricking and during the early stages of ether anesthesia. During deep anesthesia it was abolished and at such times in the absence of true paradoxical phenomena the right was usually the smaller pupil. On the 38th day cord transection between roots C iv and v failed to abolish the pseudo-paradoxical dilatation in the right pupil whilst cord transection between roots L iv and v abolished it permanently (figs. 1 and 2). The sites of both of the cord transections were verified at autopsy.

Protocol. Cat 8. Male, adult

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm	*mm*	*mm*	*mm*
	a.m.	April 9, 1921. Crushed left sciatic nerve				
38th	6:58	Pseudo-paradoxical dilatation in right eye Cutaneous hyperalgesia in right thigh and leg	6.0	4.5	0.5	0.5
	7:10	Deep ether anesthesia; wink reflex absent	8.0	8.5	2.5	2.5
	7:14	Light anesthesia; wink reflex present	9.0	8.0	2.0	2.5
	7:15	Becoming conscious	11.0	9.5	2.5	2.5
	7:18	Deep anesthesia; before adrenalin	3.0	3.0	2.5	2.5
	7:18½	Adrenalin mii True paradoxical effects absent in right eye	13.0	14.5	0.0	0.0
	7:50	Transected cord between roots C iv and v				
	8:20	Pseudo-paradoxical dilatation in right eye (fig. 1)	8.5	7.0	2.5	2.0
	8:34	Transected cord between roots L iv and v				
	8:44	Pseudo-paradoxical dilatation absent in right eye (fig. 2)	7.5	8.0	1.5	1.5

In cat 9 after crushing the right sciatic nerve results similar to these found in cat 8 were obtained. This animal was allowed to recover fully. The pseudo-paradoxical effects in the left eye and the cutaneous hyperalgesia in the right leg disappeared between the 47th and 53rd day.

Between the 53rd and 82nd day it was found that pricking either hind leg caused both pupils to dilate but in each instance the contra-lateral was the larger pupil. Throughout the period in which the left pupil exhibited pseudo-paradoxical dilatation it was noted that the rhythmic pupillary oscillations were increased in range (hippus) in the left as compared with the right pupil. So marked was the difference in this respect between the two pupils that at times it seemed that the pupillary oscillations were altogether absent in the right pupil though markedly present in the left.

After complete section of the posterior nerve roots. In cat 10 the right posterior nerve root C ii was divided. The right was the larger pupil 19 minutes after the operation but 14 hours later and daily thereafter until the 27th day it was the smaller pupil. From the 27th to the 34th day the right pupil exhibited pseudo-paradoxical dilatation. From the 35th to the 174th day the right was the smaller pupil. From the 7th to the 27th day adrenalin elicited true paradoxical effects in the right eye but failed to do so before the 7th and from the 31st to the 174th day.

After partial section of the posterior nerve roots. In cat 11 the right posterior nerve roots L v to S i were partially cut across. The left pupil exhibited well-marked pseudo-paradoxical dilatation from the 2nd to the 55th day and during this period adrenalin failed to elicit true paradoxical effects in the left eye. From the 56th to the 71st day adrenalin elicited true paradoxical effects in the left eye and during this period pseudo-paradoxical dilatation was either entirely absent or was barely perceptible. In three other animals after partial posterior root section similar results were obtained. In all these animals the rhythmic pupillary oscillations were markedly exaggerated in range in the pupil that exhibited pseudo-paradoxical dilatation.

After excision of the posterior root ganglia. In cat 12 the posterior root ganglia L vii to S ii were excised on the right side. Care was taken to avoid as much as possible injury of the left posterior nerve roots and of the cord. Posterior root L vi was accidentally divided on the right side and owing to retraction of the spinal ganglion the latter was not removed. Twenty minutes after the operation and practically throughout the whole period from the 3rd to 125th day the left was the smaller pupil. From the 19th to the 42nd day although the left was the smaller pupil before the administration of ether it became larger than its fellow during the early stages of anesthesia. From the 47th to the 125th day pseudo-paradoxical dilatation was completely absent, the left pupil being always smaller than its fellow even in the early stages of anes-

thesia. Occasionally in deep anesthesia the left pupil became larger than the right. During this period, i.e., from the 47th to the 125th day, adrenalin elicited well-marked true paradoxical effects in the left eye. On the 125th day sensibility for pricking at 27.2 grams pressure was absent on the ventral aspect of the right hind leg from the dorsum of the foot to a point a little way above the knee and on the dorsal aspect of the limb up to the level of the ischial tuberosity. Above the limit of lost sensibility an area of cutaneous hyperalgesia extended on the trunk to the level of the crest of the ilium. At autopsy regeneration was found to have taken place in the right posterior nerve root L vi and to a much less extent in posterior roots L vii to S ii.

Discussion and summary. In the resting animal the pupil that is the seat of latent pseudo-paradoxical dilatation is often smaller than its fellow and it is only when the animal is roused that the pseudo-paradoxical dilatation becomes manifest. Once evoked it tends to persist for some time. These facts show defective functioning in the pupil dilator mechanism, presumably in the injured primary sensory neurones which exhibit a slightly heightened initial threshold and a tendency to over-react once the stimulus reaches threshold value. The constancy of its occurrence after injury of the primary sensory neurones shows that pseudo-paradoxical dilatation is essentially dependent upon activities propagated within the injured neurones. Compare cats 1 and 3 in which the phenomenon was re-induced by a repetition of the original injury. The cord transections in cats 4, 7 and 8 further show that the dynamic factor of the mechanism resides in the injured primary sensory neurones whilst the results in cats 10 and 12 show that it resides in the bodies of the injured neurones. In cat 10 after complete section of posterior nerve root C ii the phenomenon was absent from the 1st to the 146th day except during a few hours immediately succeeding the operation and during a period extending from the 27th to the 34th day when the temporary pseudo-paradoxical dilatation was undoubtedly the result of minor injuries incidental to the experimental lesion proper. Compare cat 3 in which mere section of the muscles, etc., caused sufficient nerve injury to induce pseudo-paradoxical and true paradoxical phenomena. The appearance of the phenomenon just after the operation was due to excitatory effects in slightly injured neurones still in functional continuity with the pupil dilator mechanism. In cat 12 after excision of the dorsal root ganglia L vii to S ii the phenomenon was absent from the 1st to the 139th day with the exception of a period extending from the 19th to the 42nd day when it was present

only on marked excitement as during the early stages of ether anesthesia. During this period its occasional appearance was undoubtedly associated with the partial regeneration that took place in the severed posterior nerve roots and which in turn points to the existence, in the nerve roots, of neurone bodies proximal or distal to the sites of excision.

Many facts point to increased specific neural functioning on the part of the injured primary sensory neurones as the immediate cause of pseudo-paradoxical dilatation. Compare the cutaneous hyperalgesia in practically all of the animals, the exaggerated rhythmic pupillary oscillations (hippus) in cats 9 and 11, and the abolition of the pseudo-paradoxical phenomenon by certain cord transections as in cat 12. Compare also the absence of the phenomenon after complete posterior nerve root section in cat 10 and after excision of the dorsal root ganglia in cat 12. In this hyperfunctioning of the sensory neurones two forms are distinguishable: *a*, The early hyperactivity of slightly injured (over-stimulated) neurones; and *b*, the late hyperactivity of the more seriously injured neurones. The late form succeeds a period of enforced inactivity, absolute or relative, incidental to axonal reaction phenomena in the bodies of the more seriously injured neurones. The hyperfunctioning (hypermetabolism) of the neurone bodies during this stage belongs in part to the more primitive embryonic mechanism of growth and repair in the damaged neurones and in part to the more highly differentiated mechanism concerned in the elaboration and discharge of nerve impulses. Both the early and the late form of hyperfunctioning in which impulses are discharged either spontaneously or upon slight stimulation, and continue to be discharged after the stimulus has been withdrawn, seem to be of universal occurrence after continued over-stimulation or injury not only in sensory neurones but in all neurones of the dynamic type whatever be their location. Compare the abortive attempts at regeneration which may be seen within the first few days not only in the proximal but also in the distal segment after sciatic section (3). The tendency on the part of the injured primary sensory neurones to continued over-action in response to noxious stimulation seems to be a heritage from the more primitive, non-cognitive mode of protection in which, after direct noxious contact, the part is suddenly withdrawn by a neuro-muscular mechanism acting on the "all or nothing" principle. Indeed, pseudo-paradoxical dilatation itself seems to be nothing more than a manifestation on the part of the injured sensory neurones of a more primitive, less ordered mode of functioning in which readiness to respond maximally to noxious stimulation is the

fundamental pre-requisite. Compare the mechanism of the flexion reflex in which restrictive control is at a minimum being confined exclusively to inhibition of the antagonists. The two forms of hyperfunctioning represent the first and third stages of the biological response of the neurones to injury. Between these a second stage intervenes, viz., the stage of inactivity histologically characterized in the neurone bodies by well-marked retrograde degeneration (axonal reaction phenomena), i.e., impaired staining capacity of the cytoplasm, chromatolysis, and swelling distortion and lateral displacement of the nucleus. Physiologically this stage is characterized by the absence or diminution of pseudo-paradoxical dilatation and by hypersensibility of the related pupil to adrenalin (true paradoxical phenomena). In injuries such as crushing or section of a sensory nerve each of the stages of hyperfunctioning gradually merges into and overlaps the stage of inactivity so that many neurones may be in a state of heightened activity at a time when the great majority of their fellows are absolutely or relatively inactive, i.e., at the height of retrograde degeneration. The determining factor, therefore, as to whether at any given moment pseudo-paradoxical or true paradoxical phenomena shall obtain is the algebraic sum of the centripetal impulses emanating in a given time from the injured neurones as compared with the sum of the impulses emanating from the corresponding neurones of the sound side. When the neural discharges from the injured neurones overbalance the discharges from the corresponding neurones of the sound side, pseudo-paradoxical dilatation appears but where the contrary obtains true paradoxical phenomena supervene. After sciatic section or crushing the true paradoxical stage extends usually from about the 8th to the 29th day, the early pseudo-paradoxical stage extending from the 1st to about the 8th day, and the late from about the 29th day until recovery. After severe nerve injuries the recovery is seldom complete. The injured sensory neurones after going through the second stage of hyperfunctioning become quiescent and the pupil that previously had exhibited pseudo-paradoxical dilatation becomes, as it usually is in the true paradoxical stage, smaller than its fellow. Notwithstanding this apparent inadequacy of the flow of centripetal impulses, true paradoxical effects can no longer be elicited. Compare cat 9 in which after crushing the right sciatic nerve adrenalin failed to elicit true paradoxical effects in the left pupil from the 53rd to the 82nd day although during this period the left was the smaller pupil. Compare also cat 10. Following section of the lumbo-sacral posterior nerve roots the true paradoxical

stage makes its appearance very late, e.g., on the 48th day in cat 12. This seems to be the result of unavoidable injuries inflicted upon the lumbo-sacral pathways springing from the unoperated side and maximally related to the contralateral pupil. Slight injury of these could beget on its own account an early transient form of pseudo-paradoxical and true paradoxical phenomena which for the time being might overshadow the phenomena resulting from the experimental lesion proper. Compare cat 4 in which during the early post-operative period the effects of section of the muscles about the left shoulder overshadowed the effects of the nerve lesion proper.

The transections performed in cats 4, 7 and 8 indicate that the centripetal impulses immediately responsible for pseudo-paradoxical pupil dilatation pass in part to the brain, where presumably they inhibit the constrictor mechanism, and in part to the lateral grey column of the upper thoracic segments (lower cilio-spinal center of Budge) where they condition (augment in the pseudo-paradoxical stages) the outflow of pupil dilator impulses over the cervical sympathetic nerve. In the intermediate stage in which the flow of centripetal impulses from the injured sensory neurones is below par there results a diminished outflow of pupil dilator impulses via the cervical sympathetic of the related side and the corresponding pupil is, under ordinary conditions of quiet, smaller than its fellow and becomes, after a period of incubation, hypersensitive to adrenalin (true paradoxical stage). The presence of cutaneous hyperalgesia during the two stages of hyperfunctioning and its absence during the stage of impaired functioning points to the neurones of the noci-ceptive (affective) system as the ones fundamentally involved in the mechanism of both pseudo-paradoxical (hyperfunctioning) and true paradoxical phenomena (impaired or suppressed functioning). This relationship has important clinical and perhaps medico-legal significance as with a knowledge of the stages of reaction of the sensory neurones to mechanical, toxic-infective, or other injury, the size of the pupil maximally related to the injured area may furnish objective evidence as to the existence or non-existence of pain and tenderness or of tissue injury. The author has studied this matter and has found that in man the relations of the pupils to the different regions of the periphery are similar to those found by him experimentally in animals, viz., that the sensory nerves of the head, neck and upper extremity are related maximally, if not exclusively, to the homolateral pupil whereas those of the lower extremity are related maximally to the contra-lateral pupil. In injuries, therefore, of one side of the

head or neck, or of an upper extremity, the excitatory or paretic pupillary effects are to be looked for in the homo-lateral eye, whereas in injuries of the lower extremity they are to be looked for in the contra-lateral eye.

CONCLUSIONS

1. The dynamic factor in the mechanism of pseudo-paradoxical pupil-dilatation resides in the bodies of the injured primary sensory neurones.

2. The centripetal impulses immediately causing pseudo-paradoxical pupil-dilatation after sensory nerve lesions are the result of hyper-functioning of the bodies of the injured primary sensory neurones.

3. In the hyperfunctioning of the neurone bodies two forms are distinguishable: *a*, the early, representing the more or less continued hyperactivity of the slightly injured (over-stimulated) neurones; and *b*, the late, in which the hyper-activity (hypermetabolism) represents in part the more primitive embryonic mechanism of growth and repair and in part the more highly differentiated mechanism concerned in the elaboration and discharge of nerve impulses.

4. In the response of the sensory neurones to injury three stages are distinguishable of which the 1st and 3rd are characterized by hyper-functioning of the injured neurones (pseudo-paradoxical phenomena) whereas the 2nd or intermediate stage is characterized by hypofunction-ing of the injured neurones and hypersensibility of the effectors of the related pupil dilator mechanism to adrenalin (true paradoxical phenomena).

5. After sensory nerve injury the determining factor at any given moment as to whether pseudo-paradoxical or true paradoxical phe-nomena shall obtain is the algebraic sum of the centripetal impulses emanating within a given time from the injured sensory neurones as compared with the sum of the centripetal impulses emanating from the corresponding neurones of the sound side. When the impulses from the injured neurones over-balance those from the corresponding neurones of the sound side, pseudo-paradoxical dilatation ensues, but where the contrary obtains true paradoxical phenomena supervene.

6. Part of the centripetal flow of impulses responsible for pseudo-paradoxical dilatation passes to the brain where it inhibits the pupil constrictor mechanism and part impinges upon the related half of the lower cilio-spinal center of Budge where it augments the outflow of pupil dilator impulses via the cervical sympathetic nerve.

7. The neurones of the noci-ceptive (affective) system are the ones fundamentally concerned in the mechanism of both pseudo-paradoxical (hyperfunctioning) and true paradoxical (hypofunctioning) phenomena.

8. Following continued over-stimulation or injury, hyperfunctioning seems to be at certain stages a universal occurrence among neurones of the dynamic type whatever be their location.

9. Injured primary sensory neurones tend to revert to the primitive, "all or nothing" mode of responding to noxious stimulation.

10. As the pupillary conditions incidental to the two pseudo-paradoxical stages (dilatation) and the true paradoxical stage (constriction) furnish objective evidence of the presence or absence respectively of pain and tenderness or of tissue injury, they have important clinical and perhaps medico-legal significance.

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LATENT PERIODS IN THE RECIPROCAL ACTION OF ANTAGONISTIC MUSCLES

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Upon stimulation of an afferent nerve the reflex muscular response is characterized by the contraction of one group of muscles and the relaxation of their antagonists. This has been shown experimentally for a number of pairs of antagonistic muscles, e.g., the external and internal rectus muscles of the eye, etc. It has been the opinion that the contraction of the one occurs simultaneously with the relaxation of the other, and indeed this seems to be the case when the myograms of the opposing muscles are taken on a slowly moving drum (1). Two exceptions to this rule have come to our attention; one in the case of dilatation of the pupil of the eye, where the augmentation of the dilator tone appears to occur before the inhibition of pupil constrictor tone (2), the other in the case of rebound contraction and relaxation in the gastrocnemius and tibialis anticus muscles (3), to which reference will be made later.

Upon repeating certain of Sherrington's experiments on reciprocal innervation we become convinced that antagonistic muscles do not act exactly simultaneously, but that the contracting muscle begins its action earlier than its relaxing antagonist.

Methods. The experiments were performed on cats decerebrated on the Sherrington decerebrator. In marking the point on the skull where the cut was to be made in decerebration, a distance of 24 to 26 mm. was measured back of the fronto-parietal suture in the smaller cats, and 26 to 28 mm. in the larger animals. The result in each case was that the plane of section passed through the anterior corpora quadrigemina, usually in the forward portion. There was nearly always marked "decerebrate rigidity," and the preparation usually remained in good condition for over six hours.

The antagonistic muscles used were the vastus crureus (extensor) and semitendinosus (flexor). The former was left in place, and a lever attached to the stump of the lower limb, which had been amputated be-

low the knee, recorded the movements of this, the extensor muscle. The semitendinosus, however, was cut away from its insertion into the tibia and the freed tendon was connected to a lever. The muscle was then entirely loosened from its attachments to skin, other muscles, etc., up to a short distance above the knee. When this muscle was put under tension, i.e., if the lever arm were held down by an elastic band, passive raising and lowering of the stump with the resultant shortening and lengthening of the vastus crureus sometimes caused also a change in the position of the lever attached to the semitendinosus, but a change in the opposite direction. This reciprocal action in passive movement, when it appears, is not due to mechanical factors if the semitendinosus muscle is sufficiently freed, but it is a consequence of reciprocal innervation since it occurs when the semitendinosus is dissected entirely free from all other tissues as far as its attachment to the femur in the thigh, but fails to occur if the nerve to the semitendinosus is cut. We more often obtained relaxation of the semitendinosus in the crossed extension reflex if this muscle were put under considerable tension by means of an elastic band attached to the lever, or if a flexion reflex had been elicited immediately before.

In order that these two muscles might operate alone without complication from neighboring muscles, all the branches of the femoral nerve except that to the vastus crureus were severed, and likewise all the hamstring nerves except the branch to the semitendinosus were cut. In many cases the iliopsoas muscle was transected, but we found that leaving this muscle intact made no difference in our records provided the body of the cat was held sufficiently rigid. The cat was held firmly in place by a drill-pin inserted into the lower condyle of the femur, and often a second pin was screwed through the greater condyle of the femur into the pelvis, and in a few cases a third pin was inserted into the pelvis of the opposite side.

Both ipsi- and contralateral stimuli were employed. The afferent nerve was in each case the sciatic. This nerve was ligated and severed near the knee and enclosed in Sherrington electrodes for stimulation in a central direction. The source of stimulation was the interrupted current from the secondary coil of an inductorium, and records were taken on a smoked drum moving at the rate of 27.5 cm. per second.

Results. *1.* An ipsilateral stimulus elicits the flexon reflex in which the semitendinosus muscle contracts and the vastus crureus relaxes. In our earlier records it was difficult to judge the exact instant when contraction or relaxation began, but by carefully adjusting the limbs of

the preparation and then stimulating during a period of absolute quiet, the results were unmistakable. Out of approximately a hundred records the contraction of the flexor muscle occurred before the relaxation of the extensors (fig. 1). The difference in the latency of the action of the two muscles is difficult to determine accurately from myograph records since there is no abrupt change at the point of departure from the base line. As far as it was possible to judge by this method of recording, the difference in the latent periods ranged from 3 to 20 σ, with an average of about 10 σ. In 3 per cent of the cases, the two actions

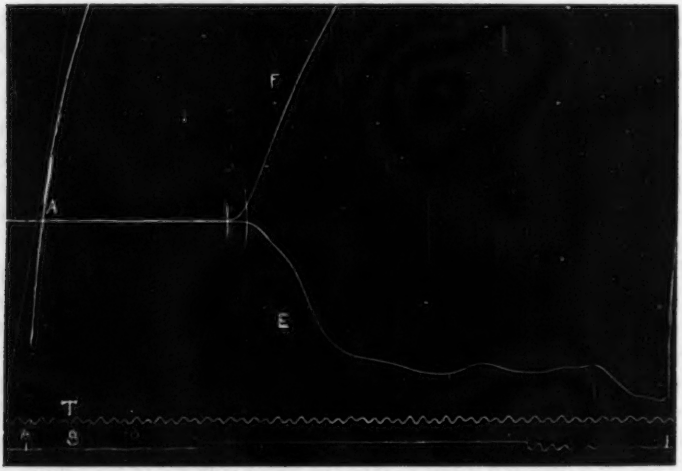

Fig. 1. *Flexion reflex.* Myograph records of contracting semitendinosus muscle; flexor, *F*, and relaxing vastus crureus muscle, extensor, *E*.

Signal *S*, marks time of stimulation of ipsilateral sciatic nerve; time *T*, in hundredths of a second; alignment marks, *A*, abscissae—show the interval between contraction and relaxation.

appeared to occur simultaneously, but never did relaxation precede contraction.

2. Contralateral stimulation causes the extension reflex with contraction of the vastus crureus muscle and in exceptionally favorable preparations with relaxation of the semitendinosus. Records of this reflex were not so uniform in results as those of the flexion reflex but still they are significant. Out of a series of approximately a hundred readings the extensor muscle contracted before the flexor relaxed in

80 per cent of the records, the two muscles acted simultaneously in 13 per cent, and relaxation occurred before contraction in only 7 per cent. The difference in latency in the first group showed the same range and average as in the flexion reflex.

The flexion reflex and the extension reflex employ the two muscles in exactly the opposite manner, yet in both reflexes it was practically always the contracting muscle which acted first, regardless of whether it was the flexor or extensor (fig. 2).

Fig. 2. *Crossed extension reflex.* Myograph records of contracting vastus crureus muscle, *E*, and relaxing semitendinosus muscle, *F*.
Signal, *S*, marks time of stimulation of contralateral sciatic nerve.

3. Sherrington (1) has shown that one reflex may be inhibited by another. If while stimulating the contralateral sciatic nerve, consequently with the leg in a state of extension, the ipsilateral nerve is stimulated with a sufficiently strong stimulus, the leg at once flexes. Similarly, the flexion reflex may be replaced by the extension reflex. Records of this change from extension to flexion and vice versa show

again that the contracting muscle acts first and with similar time relations as in the flexion reflex. Thus, the rule is followed no matter whether the reflex is initiated from rest or whether it appears while another reflex is actually in operation (fig. 3).

4. In rhythmic movements during stimulation we find that contraction precedes relaxation in each successive movement (fig. 4).

5. After the cessation of a stimulus causing either flexion or extension there is a rebound of the muscle in the opposite direction which often lasts for several seconds. The phenomena of rebound have been dealt with at length by Graham Brown (3) and Forbes (4). We find that relaxation occurs *before* contraction in rebound after stimulation (fig. 5). For example, if an ipsilateral stimulus has been sent in and this has caused flexion of the leg through contraction of the semitendinosus and relaxation of the vastus crureus, there occurs upon the cessation of the stimulus a rebound extension in which the semitendinosus relaxes and the vastus crureus contracts—*and the semitendinosus acts first.* This is apparently the exact opposite of what we have found in all the other cases cited, but we believe that this follows of necessity from the arrangement of neurones which we think is demanded for reciprocal innervation of antagonistic muscles.

6. One phenomenon should be recorded before passing to a consideration of the theoretical implications of our results.

We have found in the course of our work some four cats which, so far as one could judge, were prepared in exactly the same way as all the other animals, three of which, nevertheless, showed only flexion of the leg no matter whether contra- or ipsilateral afferent nerve was stimulated, while the fourth gave only the crossed extension reflex. This reflex reversal has been termed by Graham Brown (3) the "dilemma of reaction." Sherrington (5) found that with the extensor muscle reversal depends upon the length of the muscle, that is, the position in which the leg is passively placed by the experimenter, and Forbes (6) showed that it is also brought about by actively maintained posture. Thus, if the leg is placed in extreme flexion, any stimulus is more likely to result in extension, hence an ipsilateral stimulus may give not flexion but extension. This result is the more likely to occur if there is little or no decerebrate rigidity. This was the case in the one cat mentioned above. The converse is also true, for if decerebrate rigidity is marked, any stimulus may result in flexion. This was the case in the three cats. In these cases of reflex reversal we found the inhibited muscle to have the same relative latency that it would have had if the reflex had been produced by the normal stimulus.

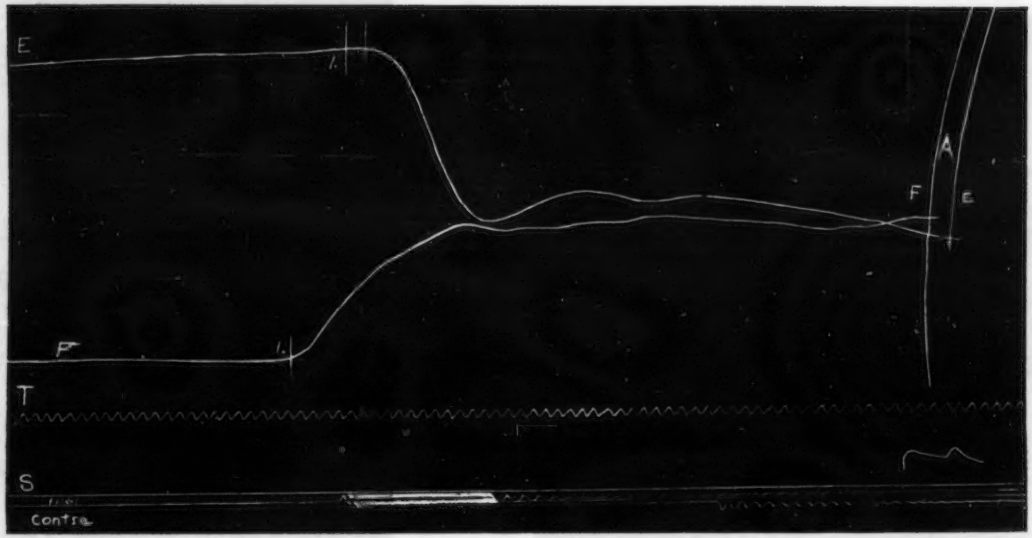

Fig. 3. *Crossed extension reflex replaced by flexion reflex.* Myograph records of contracting semitendinosus muscle, *F*, and relaxation of vastus crureus muscle, *E*.

Signal, *S*, lower signal contralateral stimulation causing crossed extension reflex. Stimulus thrown in before the beginning of this portion of myogram. Upper signal ipsilateral stimulation causing flexion reflex to replace the crossed extension reflex.

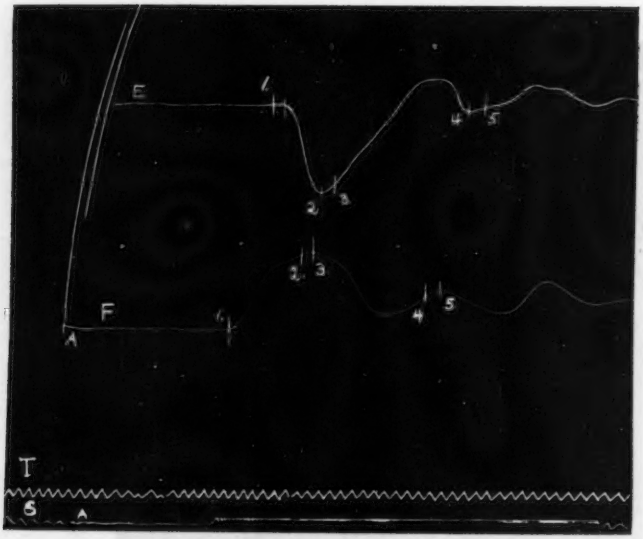

Fig. 4. *Rhythmic contractions during stimulation.* Myograph records of contracting semitendinosus muscle, *F*, and relaxing vastus crureus muscle, *E*, and vice versa.
Signal marks time of stimulation of ipsilateral sciatic nerve.

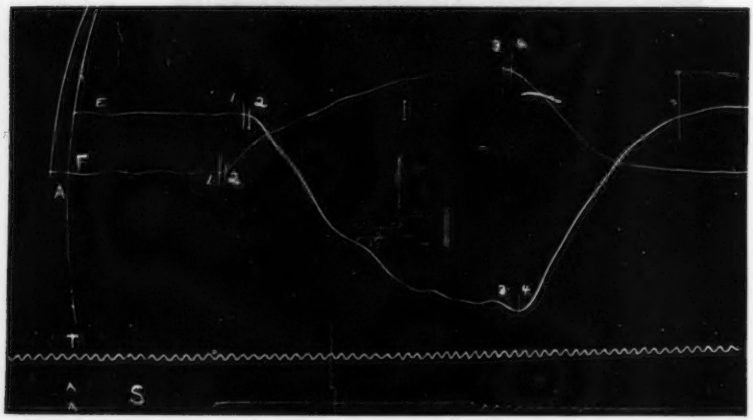

Fig. 5. *Rebound.* Myograph records of contracting semitendinosus muscle, *F*, and relaxing vastus crureus muscle, *E*, with rebound.
Signal marks time of stimulation of ipsilateral sciatic nerve.

Discussion. The simplest scheme to account for the facts of reciprocal innervation and the action of antagonistic muscles is that proposed by Sherrington (1). According to this scheme the afferent nerve divides into branches which form synapses with the motor neurones of each of the antagonistic muscles, inhibiting the one, exciting the other. The work of Graham Brown (3) on rhythmic movement shows that the controlling factors do not lie entirely on the afferent side of the reflex arc, but that the motor centers themselves are involved. This is an elaboration of an alternative hypothesis which Sherrington had already advanced. The difference between the two hypotheses lies in the assumption that in the latter case one motor center is able to inhibit the

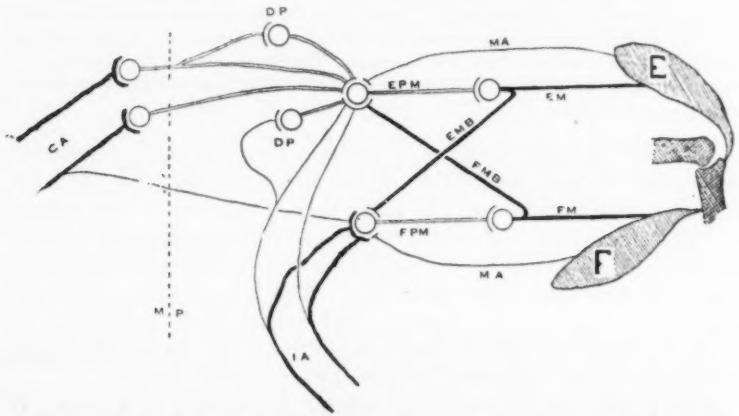

Fig. 6. Diagram of spinal neurones and their connections to provide a possible basis for analyses of reflexes. *E*, extensor muscle; *F*, flexor muscle; *MP*, median plane; *C A*, contralateral afferent fibers, branch indicated by light line does not normally conduct effective impulses; *I A*, ipsilateral afferent fibers, branches indicated by light lines do not normally conduct effective impulses; *D P*, delay paths, representing extensive central connections to provide for prolonged after-discharge; *E P M*, extensor pre-motor neurone; *F P M*, flexor pre-motor neurone; *E M*, extensor motor neurone; *F M*, flexor motor neurone; *E M B*, collateral branch of extensor motor neurone; *F M B*, collateral branch of flexor motor neurone.

action of the other. In other words, there appears to be a cross connection between the two motor centers which is responsible for the relaxation of one of the antagonistic muscles.

Forbes (6) has carried this scheme further in an attempt to explain central phenomena of "neural balance," etc., in terms of the principles

of muscle-nerve physiology worked out by Lucas and Adrian. These principles involve the all-or-none law, and especially the so-called Wedensky inhibition. Forbes' scheme is a combination of the hypothesis of divided afferent nerve and connected motor centers. A modification of his diagram reproduced in figure 6 illustrates the possible arrangement of neurones.

> If we assume that the collateral branch of the motor neurone leads to the pre-motor neurone of the antagonistic center we shall at once establish the condition for converting excitation into inhibition in that center. Thus, if a pre-motor neurone in the extensor center is at a given moment conducting impulses with a frequency below the critical value, and therefore causing excitation in the motor neurone, then if a series of impulses is set up in the flexor motor neurone, these will by virtue of the collateral branch leading to the extensor pre-motor neurone, set up in it additional impulses which will raise the frequency therein above the critical value, and inhibition will result. The same picture may be reciprocally applied to the inhibition of flexors through activity in the extensor motor neurones. (6, p. 304.)

Now if one compares the nervous pathway involved in this scheme for the impulse causing contraction of the flexor muscle with that for relaxation of the extensor, one finds that the latter is the longer, necessitating at least one more neurone and synapse than the former. Since there is always a delay at a synapse one would expect relaxation to occur slightly later than contraction. This has been the rule in our experiments. Our results therefore lend weight to the correctness of the motor-center hypothesis, that is, inhibition is due to impulses passing from one motor center to the other by a collateral branch.

There is one difficulty with the scheme as outlined by Forbes. If the divided afferent neurone carries impulses to each pre-motor center, then the first action should be simultaneous contraction of both muscles. Meanwhile impulses would be sent back over each collateral branch to both pre-motor neurones, and both muscles should now be inhibited. This, of course, is not the case at all. If, however, it is supposed that under normal circumstances an impulse along an afferent path affects only one of the two pre-motor neurones, there is present all the mechanism necessary for the contraction of one muscle and the relaxation of the other, and one would expect the latter action to occur slightly after the former. In order to prevent the impulse from passing to both pre-motor neurones it may be supposed that the block is complete at the synapse between the branch of the afferent nerve and the pre-motor neurone which is ordinarily inhibited.

Nevertheless there must be connections of the afferent nerves with the pre-motor centers of both antagonistic muscles. This is demanded by the phenomena of "dilemma of reaction" and in rhythmic movements during continued stimulation. Reversal may take place in the following manner: in a cat with pronounced decerebrate rigidity or with the extensor muscle greatly shortened, contralateral stimulation results in flexion because the constant flow of impulses to the extensor pre-motor neurone has raised the resistance over this pathway to such an extent that the impulse is now able to cross the synapse to the flexor pre-motor center where under ordinary circumstances there would have been a complete block. One would expect, if this were the case, that the contracting muscle again would act first, and this is borne out in our experiments. The explanation of the rhythmic character of contractions during stimulation is probably similar to that given by Sherrington (1) to account for the "mark-time" and other alternating reflexes. Fatigue at the excited motor center increases the resistance to the passage of nervous impulses and finally extinguishes them. In the meanwhile the excitability in the inhibited motor center has been raised so that, as a result of "successive spinal induction," the opposed or antagonistic act is produced, and the inhibited muscle now contracts. Here as well as in the "dilemma of reaction" afferent neurones must be capable of affecting both motor centers.

In rebound contraction and relaxation, viz., the contraction and relaxation which occur immediately after stimulation has ceased, we have found, however, that relaxation precedes contraction. Nevertheless this apparent contradiction of our other results is consistent with Forbes' scheme and may be explained as follows: the inhibiting impulses which have been started over the collateral branch along the longer pathway, for example to the extensor muscle, may continue to act after the last impulse has reached the flexor muscle. Thus the flexor muscle will begin to relax before the inhibiting impulses to the extensor have left this pathway open for impulses causing contraction.

SUMMARY

1. In the reciprocal action of antagonistic muscles as studied in the flexion and crossed extension reflexes, contraction occurs slightly before relaxation.

2. This relationship also holds when one reflex is replaced by the other, in the so-called "dilemma of reaction," and in rhythmic movements during stimulation.

3. The reverse is true in the phenomena of rebound.

4. These results are in perfect accord with the "motor center" theory which assigns to a collateral branch between the motor centers the function of inhibition.

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STUDIES ON THE ORIGIN AND CONDUCTION OF THE CARDIAC IMPULSE

VIII. The Permanent Rhythm Following Destruction of the Sino-Auricular Node

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A number of investigators have shown that when the normal function of the sino-auricular node as the seat of impulse initiation is temporarily or permanently destroyed, a new type of cardiac rhythm develops in which the auriculo-ventricular sequence disappears, or in which the interval between the auricular and ventricular beat is much shortened. In the former case, the new seat of impulse initiation has been located in the main portion of the auriculo-ventricular node, while rhythms of the latter type have been shown to have their origin, in most instances at least, in the extension of this node around the mouth of the coronary vein, the so-called coronary sinus region. The literature of the rather extensive work in this subject has been reviewed in detail by the present writers (1), and reference may be made to this publication for further details and for the bibliography. All of this work, so far as we know, has been carried out only in the usual short experiment, and no one has studied the later effects of sino-auricular node ablation and the type of final or permanent rhythm established. Criticism of the interpretation of experiments previously reported in favor of the paramount importance of the node for the normal cardiac rhythm has also been offered, in that the occurrence of the abnormal rhythm may be a temporary matter due to inhibitory influences (2). Finally, the relation of the auriculo-ventricular node to chronotropic vagus control is a matter of some dispute. Thus Lewis (3) states that the vagus exerts its principal characteristic control on heart rate on the auriculo-ventricular node rather than the sino-auricular node, while Schlomovitz, Eyster and Meek (4) obtained experimental evidence in favor of a greater control upon the sino-auricular node. It was with the idea of throwing further light on these matters that the present research was undertaken.

Methods. Dogs were used in all the experiments. Electrocardiagrams were obtained for two or three days previous to the operation on the node and in certain cases the influence of exercise and of atropinization as controls on the normal animal were also studied. The normal resting heart rate and auriculo-ventricular interval (P R electrocardiographic interval) were thus obtained. The animal was then morphinized and prepared for the operation which was carried out aseptically under ether. In seven animals the sino-auricular node was tied off from the surrounding auricular and caval regions by a series of ligatures passed through the tissue and tied. In ten animals the node was first isolated by a series of mattress sutures and then excised.

The operative procedure follows. The right lateral chest from the clavicle to the sixth rib is carefully shaved and iodine and alcohol applied. Artificial respiration is carried out by means of a rubber tube passed to the tracheal bifurcation and connecting through the ether bottle with an interrupted air blast. An incision is made following the line of the third rib and extending from the mid-axillary to the mid-clavicular lines. The underlying portion of the third rib is carefully resected, freed by means of a curved dissecting probe from the periosteum and about 5 cm. of the bone removed, leaving the pleura intact. The pleura is now incised and small retractors placed at right angles in the wound. This gives an oval shaped opening through which the caval regions and right auricle are seen. The pericardium is slit for a distance of several centimeters along the line of the sulcus terminalis and the edges brought out and sewed temporarily to the edges of the opening in the thoracic wall. A single ligature is now passed superficially through the fat along the lower caval auricular boundary and brought out through the anterior chest wall close to the right edge of the sternum in the sixth or seventh interspace. Traction on this ligature rotates the heart to the left and brings the sulcus terminalis into the center of the opening. A head mirror with self-contained light is necessary to properly illuminate the field of operation. The borders of the node on the caval and auricular sides are now interrupted by a series of silk ligatures, or if the node is to be excised, a series of mattress sutures is laid, beginning well above the head of the node in the angle between the superior vena cava and auricular appendage and extending to the lower sulcus. Temporary "holding stitches" may be laid above and below the node and brought out to the upper and lower edges of the opening in the thoracic wall and will facilitate the placing of the sutures. Beginning at either the upper or lower end a suture is passed

from the caval side under the node and brought out on the auricular side. It is then carried up or down for a half centimeter or less and passed through in the opposite direction, namely, from the auricular side under the node and out through the caval side. The two free ends thus project from the caval borders, and when tied bring the auricular and caval tissue together leaving the tissue containing the node in the form of a fold projecting outward. A second ligature is laid in a similar manner contiguous to the preceding, and successive sutures are thus placed until the whole area of the node is folded up and sewed off from the surrounding tissue. Small curved needles of several sizes, a good needle holder and small fine pointed hemostats facilitate this procedure. After completion of the process of laying and tying the sutures, the tissue contining the node and overlying the approximated auricle and caval tissue is excised by means of curved scissors. If carefully done, none or only a few drops of blood are lost. Occasionally small hemorrhages occur around the sutures, but these are easily controlled by applying pressure for a moment with gauze. After excision of the node the holding stiches are removed and the pericardium closed by a single line of sutures. The pleura is now brought together by a line of sutures and before the last one is tied the lungs are inflated by a continuous blast of air, the air being thus largely removed from the intrathoracic cavity. The thoracic muscles are now approximated by sutures and finally the wound is closed by a row of subcutaneous and a continuous skin suture.

Of the seventeen animals operated upon, fourteen made uneventful recoveries. Three died on the fourth or fifth day from penumonia, in one pericarditis with effusion was also present. Some trauma to the lung near the site of the operation is almost unavoidable and the long exposure and difficult operation probably lowers resistance to infection. It is probable also that the inefficient cardiac action following abolition of the normal rhythm plays a rôle in lowered resistance. It seems to us significant in this respect that two of the three animals which died continued in auriculo-ventricular rhythm up to the time of death. One of these showed simultaneous beating of auricles and ventricles, while one showed a reversal of sequence, the ventricles contracting several tenths of a second before the auricles. Of the fourteen animals that survived, determination of the seat of impulse origin was made in twelve at periods from 48 hours to 97 days by opening the thorax under artificial respiration and ether anesthesia and determining the seat of initial negativity by means of pairs of electrodes connected to the string

galvanometer. Points in the sulcus above and below the excised region, the right auricle, caval region, coronary sinus and the main portion of the auriculo-ventricular node were compared in the usual manner (1). While previous experience has convinced us that the seat of impulse formation can be inferred with considerable accuracy from the length of the As-Vs interval, we utilized the opportunity presented by these experiments for direct determinations in order that our results might be more convincing, and to obtain further evidence upon the question of localization of the impulse initiation by the length of the As–Vs interval.

In the last seven experiments the tissue excised was sectioned and examined histologically to determine the amount of nodal tissue removed. At the death of the animal a block of tissue surrounding the area of excision was also saved, so that if all of the node could not be identified in the tissue originally removed, examination of the surrounding tissue would give information in regard to the amount of nodal tissue remaining.

. Effects of partial isolation and of exision of the sino-auricular node.

Table 1 gives the presence or absence of auriculo-ventricular rhythm (with zero P R intervals), the approximate time of the first appearance of the permanent rhythm, the duration of observation and the seat of impulse formation in the permanent rhythm as determined by initial negativity in the exposed heart.

In the first seven experiments the tissue containing the node was ligatured off from the surrounding auricular and caval tissue. In the remaining ten experiments the node was excised. Histological control in the last seven experiments showed that the node had been completely removed in five, while in two (expers. 13 and 15) a very small amount, less than one-tenth of the total, remained. In addition to the experiments noted in the table, two animals were operated in which a section of the right auricle, contiguous to the node, was removed, the usual procedure being carried through throughout. These showed no disturbance whatever in ryhthm for the periods of observation, which were twenty-three and thirty days respectively.

The first animal noted in the table, in which an attempt was made to isolate the node by ligatures, showed no change from the normal rhythm. Of the remaining six (expers. 2 to 7 inclusive) in which similar attempts were made, one died (exper. 7) following a period of auriculo-ventricular rhythm with subsequent development of a new

rhythm with shortened P–R interval and presumably of coronary sinus origin, one survived with permanent coronary sinus rhythm (exper. 2) and the remaining three (expers. 4, 5 and 6) returned to a normal sino-auricular rhythm after several days in which auriculo-ventricular rhythm with P–R intervals of zero was present. Two of these (expers. 5 and 6) showed a period between the auriculo-ventricular rhythm and the reëstablishment of sino-auricular rhythm in which sino-auricular block was apparent, as evidenced by the usual criterion of dropped

TABLE 1

EXPERIMENT	NORMAL P-R INTERVAL	A-V RHYTHM PRESENT	PERMANENT RHYTHM ESTABLISHED	P-R INTERVAL IN PERMANENT RHYTHM	DURATION OF OBSERVATION	SEAT OF IMPULSE FORMATION IN PERMANENT RHYTHM
1	0.13	No			46 days	Sino-auricular
2	0.13	Yes	18 hours	0.10	48 hours	Coronary sinus
3	0.09	Yes	22 hours	0.08	23 days	Sino-auricular
4	0.095	Yes	6 days	0.09	38 days	Sino-auricular
5	0.11	Yes	8 days	0.12	43 days	Sino-auricular
6	0.13	Yes	3 days	0.13	38 days	Sino-auricular
7	0.11	Yes	27 days	0.09	Died 5th day	
8	0.13	Yes	9 days	0.09	64 days	Coronary sinus
9	0.11	Yes	6 days	0.07	64 days	Coronary sinus
10	0.11	No		0.07	97 days	Coronary sinus
11	0.11	Yes	7 days	0.09	10 days	Coronary sinus
12	0.11	Yes	43 days	0.085	72 hours	
13	0.105	Yes	128 hours	0.085	8 days	
14	0.09	Yes	Continued in A–V rhythm		Died 5th day	
15	0.09	No		0.07	5 days	Coronary sinus
16	0.085	Yes	Continued in A–V rhythm		Died 5th day	
17	0.10	No		0.07	7 days	Coronary sinus

beats in an otherwise regular rhythm. In one (exper. 5) this condition lasted for six days, in the other (exper. 6), it was noted in several observations over a period of nineteen hours.

Five of the seven animals in which an effort was made to isolate the node by ligatures thus survived with a normal rhythm. In four the reëstablishment of the normal rhythm was preceded by a period of auriculo-ventricular rhythm. Numerous observations made during this period showed that the reëstablishment of sino-auricular rhythm was a gradual process and that it required a considerable period for

sufficient recovery of the node or conducting paths to occur in order that the node could again serve in its full capacity as the seat of impulse formation for the heart. The second animal showed an apparent abrupt transition from auriculo-ventricular to coronary sinus rhythm in eighteen hours subsequent to the operation, as assumed from the change in P–R interval, and confirmed at the end of forty-eight hours by determination of the seat of initial negativity. The third animal showed a similar apparently abrupt transition from auriculo-ventricular rhythm to sino-auricular rhythm in twenty-two hours. In the fourth, fifth and sixth experiments, however, the transition was gradual. The fourth animal showed periods of apparently normal rhythm alternating with periods of auriculo-ventricular rhythm for sixty-six hours following the operation. The sino-auricular rhythm would continue for a variable number of beats, then following a long diastolic pause, a series of auriculo-ventricular beats at a slow rate would occur, followed again by a series of normal beats. The proportion of normal cycles increased and on the sixth day all auriculo-ventricular cycles had disappeared. This condition persisted, and thirty-two days later the seat of impulse formation was proven in the sino-auricular node by the method of initial negativity. The fifth animal showed a similar condition of a most interesting nature lasting up to the fifth day following the operation. There were periods of apparently normal rhythm in which the P–R interval gradually increased, then occurred a long diastolic pause, lasting as much as five seconds, in which the whole heart was quiescent, followed by a series of beats of auriculo-ventricular origin and finally a series of beats with gradually increasing P–R interval again appeared. On the fifth day partial sino-auricular block was evident with the dropping of every third beat. On the sixth day every fourth beat was dropped and all auriculo-ventricular beats had disappeared. This continued until the eighth day when a regular beat with normal P–R interval was established and which continued until the forty-third day, when examination with open chest proved that the impulses were arising in the sino-auricular node. The sixth experiment was similar. There was a transitory period of pure auriculo-ventricular rhythm followed by 3 to 2 and 4 to 2 sino-auricular block until the third day, when a normal rhythm had been established. This persisted until the thirty-eighth day and was then proven to be of normal sino-auricular origin.

These results in our opinion point to two conclusions of interest. In the first place they indicate that the sino-auricular node is a structure which will function after considerable injury and probably partial

destruction from interruption of its blood supply and physiological separation from contiguous tissue. In the second place they fall in line with the results from previous experiments, indicating that the paths of conduction from the node to the auricles and ventricles are diffuse, or at least that conduction by diffuse paths is possible. The period during which abnormal rhythms were present and during which a normal sino-auricular rhythm was gradually being reëstablished was associated either with a gradual resumption of function by the node or a gradual restoration of paths of conduction. Probably the first phase of recovery included the former, while the periods of sino-auricular block represented the gradual reëstablishment of functional connection with the auricles and ventricles. It would seem evident that while the heart is capable of beating fairly efficiently when under the dominance of the lower centers of impulse formation, every effort is made to secure a return to a sino-auricular rhythm, and that this will ultimately occur even though damage to the node and connecting paths has been considerable. Unfortunately we did not make histological examinations in those experiments, but there was macroscopic evidence of considerable destruction of tissue in the region ligated and organization with scar tissue formation.

Of the ten experiments in which the sino-auricular node was excised (exper. 8 to 17 inclusive) all but three (expers. 10, 15 and 17) developed auriculo-ventricular rhythm noted at the first examination subsequent to the operation and persisting in whole or in part for a period varying between thirty minutes and nine days. Two animals died (expers. 14 and 16) on the fifth day while still in this rhythm. Three animals (expers. 10, 15 and 17) showed a regular rhythm with shortened P–R interval at the first examination subsequent to the operation, a rhythm later proven to have its origin in the region of the coronary sinus. It is possible that there may have been auriculo-ventricular rhythm in those three animals in the interval between the removal of the node and the first electrocardiagram after operation, although in experiments 10 and 15, at least, this period must have been very short, since an electrocardiagram was made thirty minutes after excision. Of the five animals showing auriculo-ventricular rhythm and surviving (expers. 8, 9, 11, 12 and 13) all subsequently developed a permanent regular rhythm with P–R intervals definitely shorter than normal. The normal P–R interval in these five animals averaged 0.113 second, shortening to an average of 0.084 second or a decrease of 0.03 second with the establishment of the permanent rhythm subsequent to the operation.

In four of these animals (expers. 8, 9, 11 and 15) the seat of impulse inititation in the permanent rhythm was proven to be in the coronary sinus region by determination of initial negativity. These determinations were made on the sixty-ninth day of the experiment in two animals and on the tenth and fifth day in the other two. In view of our previous experience quoted in preceding publications (1) as well as the similar character of the results in experiments 12 and 13 to the other experiments, we feel confident that the permanent rhythm established in these two animals also had its origin in the coronary sinus region. We conclude therefore that when the sino-auricular node is excised in whole or in large part, that if the animal survives, a heart rhythm develops which has its origin in the coronary sinus area and probably in the coronary sinus portion of the auriculo-ventricular node (5). In most cases the establishment of this permanent rhythm is preceded by a period of variable length in which the rhythm originates in the main portion of the auriculo-ventricular node and which is characterized by As–Vs intervals at or approximating zero or finally in a reversal of the normal auriculo-ventricular sequence.

We have seen that the reëstablishment of a normal sino-auricular rhythm after partial isolation of the node is usually a gradual process. Our data indicate that the complete establishment of coronary sinus rhythm after excision of the sino-auricular node likewise occurs gradually in most cases. In the first experiment in which the node was excised (exper. 8) auriculo-ventricular rhythm was present within one-half hour after excision and persisted for twenty-seven hours. From this period until the ninth day, when the final coronary sinus rhythm was permanently established, there were alternating periods of auriculo-ventricular rhythm and coronary sinus rhythm or cycles of the character of each appeared in the same record. In forty-eight hours after excision an apparent coronary sinus rhythm was present with P–R intervals 0.04 second shorter than the normal. On the fourth day uninterrupted auriculo-ventricular rhythm was again present. On the fifth day a P–R interval of 0.07 second (0.06 second shorter than the normal) was present. From the sixth to the eighth day occasional beats with zero P–R intervals were interspersed in this rhythm. On the ninth day and following a regular rhythm with a P–R interval 0.04 second shorter than normal for this animal was established and continued until the final and sixty-fourth day of observation. In experiment 9, one-half hour after exercise of the node, a pure auriculo-ventricular rhythm was present. Subsequently beats of auriculo-ventricular origin were mixed

with cycles in which the P–R interval approached but did not equal the normal. The proportion of the former beats became gradually less until a regular rhythm was established on the sixth day, composed of beats of the latter type (P–R interval of 0.07 second, 0.04 second shorter than normal). In experiment 10, a regular rhythm of coronary sinus origin was established within one-half hour after excision of the node, persisting until the end of the period of observation on the ninety-seventh day. In experiment 11, six hours after the excision, most of the P–R intervals were zero, but occasional cycles with Vs-As sequence (equal to 0.03 second) were encountered and also occasional cycles with P–R intervals of approximately half the normal. This condition persisted until the twenty-fourth hour and from this time until the seventy-ninth hour a pure auriculo-ventricular rhythm was present. At the seventy-ninth and one hundred and sixteenth hour the former mixed rhythm was again present, but with occasional cycles with P–R intervals only 0.02 second shorter than the normal. On the seventh day the rhythm was composed entirely of these latter beats, which persisted for three days longer, when it was proven to be of coronary sinus origin by determination of initial negativity. In experiment 12, a pure auriculo-ventricular rhythm was present at the third, seventh and tenth hour succeeding excision of the node. At the sixteenth hour occasional beats with a P–R interval 0.03 to 0.04 second shorter than the normal were present and at the twenty-ninth hour and subsequently until the end of the experiment at the seventy-second hour, all of the cycles were of this type. In experiment 13, pure auriculo-ventricular rhythm was present until the tenth hour, when occasional cycles appeared with P–R intervals 0.04 second shorter than normal. At the sixteenth hour, these had increased in number until about one-half of the cycles were of this type, the remainder of auriculo-ventricular origin. A similar condition persisted until the hundredth hour when all the cycles were again of auriculo-ventricular origin. Eight hours later (fifth day) a regular rhythm with P–R interval 0.02 second shorter than normal had been established, persisting until the end of the experiment on the eighth day. In experiment 14, up to the seventh hour cycles with zero P–R intervals only were present. There then appeared occasional cycles with P–R intervals of 0.05 to 0.07. This continued up to the time of death on the fifth day. In experiment 15, the first electrocardiagram made three hours after excision of the node showed a regular rhythm with P–R intervals of 0.07 second or 0.03 shorter than the normal. This continued until the end of the period of obser-

vation on the seventh day when the rhythm was proven to arise in the coronary sinus region.

The cardiac rate in auriculo-ventricular and coronary sinus rhythms and the influence of exercise and atropine. The resting heart rate was reduced in all cases with the development of auriculo-ventricular or coronary sinus rhythm. The lowest heart rate was usually encountered in the former, and as the rhythm changed to the coronary sinus type an increase resulted. The average resting rate before operation was 115, during auriculo-ventricular rhythm 60 and during coronary sinus rhythm 86 per minute. The onset of auriculo-ventricular rhythm thus reduced the rate on an average of 52 per cent of the normal, returning to 74 per cent of the normal with the development of coronary sinus rhythm. The importance of these figures is in reference to the relative automaticity of the three main automatic centers of the heart, namely, the sino-auricular node, the main portion of the auriculo-ventricular node ("ventricular node") and the coronary sinus portion of the auriculo-ventricular node ("auricular node"). In a previous series of experiments (6) we obtained values of 67 and 71 per cent respectively in terms of automaticity of the sino-auricular node. These were in the usual short experiment, however, and we regard the present data as more reliable, since it was obtained under conditions in which various disturbing factors such as exposure of the heart and anesthesia were absent.

The usual result of excision of the sino-auricular node, namely, the development of auriculo-ventricular rhythm for a variable period of time with the subsequent establishment of coronary sinus rhythm as the permanent rhythm, is somewhat difficult to understand in certain respects. If the coronary sinus region has higher automaticity than the main portion of the auriculo-ventricular node as the rates in the two rhythms would indicate, it is difficult to understand why the seat of impulse initiation does not move to this region in all cases at once. This region is sufficiently far removed from the site of operation to avoid any reasonable probability that it is injured during excision of the sino-auricular node. There is no question that during the stage of auriculo-ventricular rhythm the heart is at a mechanical disadvantage. The animals are lethargic and with slight exertion they become cyanotic and dyspneic. The development of coronary sinus rhythm is associated with a distinct improvement and the animal may seem and behave quite normally. We have no explanation to offer at present for the usual transitory occurrence of auriculo-ventricular rhythm.

The influence of graded exercise (two minutes running) in producing cardiac acceleration before and after excision of the sino-auricular node was studied in three animals (expers. 8, 9 and 10). The acceleration in pulse rate as a result of exercise was reduced in all. The greatest reduction occurred during the period of auriculo-ventricular rhythm in experiments 8 and 9. The acceleration after exercise in these animals during auriculo-ventricular rhythm was scarcely perceptible and amounted to only a few beats per minute. After the establishment of coronary sinus rhythm in these two animals both the actual increase and the increase in percentage of the resting rate was also much re duced below the normal, although the resting rate was lower than before excision of the node. This condition persisted up to the last determinations made on these animals, sixty-two and fifty-three days respectively following the operation. In experiment 10, which showed no period of auriculo-ventricular rhythm, there was moderate acceleration from exercise subsequent to excision of the node, but considerably less than normal. An average normal rate of 140, reached 220 during exercise before operation. Subsequent to excision of the node and the development of coronary sinus rhythm, several determinations gave an average resting rate of 100, increasing to 142 during exercise. It seems clear, therefore, that elimination of the sino-auricular node and the presence of a rhythm arising in lower regions of automaticity is associated with greatly reduced cardiac acceleration from muscular activity. When the dominant rhythm is arising from the ventricular portion of the auriculo-ventricular node this power of acceleration is greatly reduced or nearly absent. Since Meek and Gasser (7) have shown that cardiac acceleration in exercise occurs mainly through the vagus mechanism, the reduced acceleration in auriculo-ventricular rhythm is explicable on the basis of relatively slight chronotropic vagus control over the auriculo-ventricular node. The auricular portion of this node (coronary sinus) is apparently under a greater degree of chronotropic vagus control than the main portion (ventricular portion) of the node.

In order to test further the matter of the chronotropic vagus control over these regions, the degree of cardiac acceleration following paralysis of the vagi by atropine was determined in four experiments (expers. 8 to 11 inclusive). In each case the actual as well as the percentage acceleration was markedly reduced following excision of the node, and this reduction was greater in auriculo-ventricular than in coronary sinus rhythms. As an example, in experiment 8, before operation the resting

heart rate was 120 per minute, accelerating to 288 and 340 per minute in two trials as a result of full atropinization. Twenty-four hours after excision of the node when auriculo-ventricular rhythm was present, the rate was 82, accelerating to 120 per minute after atropine. On the fifteenth day after operation when coronary sinus rhythm was present, the rate was 98 per minute, accelerating to 164 per minute after atropine administration. On the sixty-second day subsequent to excision of the sino-auricular node, with approximately the same resting rate, acceleration to 172 per minute occurred as a result of vagus paralysis.

SUMMARY AND CONCLUSIONS

The experiments reported in this communication comprise a study of the changes in cardiac rhythm for periods of days or weeks following ligation or excision of the sino-auricular node in dogs. Disturbance of the normal rhythm, as manifested by a reduction in cardiac rate, a reduction in the As–Vs interval and frequently other changes, invariably results. In the ligation experiments, recovery of the normal rhythm may ultimately occur after a prolonged period of auriculo-ventricular or coronary sinus rhythm, and in some cases after a period of partial sino-auricular block. After complete or nearly complete excision of the node the final permanent rhythm arises from the coronary sinus region, probably from the auricular portion of the auriculo-ventricular node. The presence of this rhythm was substantitated in most experiments by the initial negativity method. The development of coronary sinus rhythm is in most cases preceded by a period in which the dominant rhythm arises from the ventricular or main portion of the auriculo-ventricular node. Control experiments in which similar operative procedure was carried out and a segment of the right atrium near the sino-auricular node was excised, were without effect on the cardiac rhythm. These facts, in our opinion, tend to substantiate the paramount importance of the sino-auricular node as the seat of origin of the cardiac impulse in the normal animal. They further indicate the ability of this structure to function, even though subject to considerable injury and partial isolation, if sufficient time for recovery is allowed.

A study of the cardiac acceleration preceded by muscular exercise and by vagus paralysis under atropine administration, before and after excision of the sino-auricular node, lends further evidence to the view that it is upon this structure that the vagus produces normally its greatest chronotropic influence and through which the normal acclera-

tion in muscular exercise is in large part brought about. Similar determinations made in animals in auriculo-ventricular and in coronary sinus rhythm indicate that the vagus exerts a greater chronotropic control upon the coronary sinus region than upon the main portion of the auriculo-ventricular node. The influence of the vagus in the control of automaticity is thus greatest upon the sino-auricular node, next upon the auricular portion of auriculo-ventricular node and next upon the ventricular portion of this latter node.

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EXPERIMENTS ON THE ORIGIN AND CONDUCTION OF THE HEART BEAT

IX. Sino-Ventricular Conduction

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It is our desire in the present communication to review the work already published regarding conduction of the cardiac excitation from the sino-auricular node to the right auricle and auriculo-ventricular node and to add recent experimental observations of our own bearing on this problem.

Consideration of work already published. Evidence for separate conducting paths in the mammalian heart from the sino-auricular node to the right auricle and auriculo-ventricular node was first furnished by the present authors (1). By means of an indifferent electrode on some part of the body of the dog and another electrode placed successively on the sino-auricular node, auricle and auriculo-ventricular node, and connected with a string galvanometer, the time at which electro-negativity developed in each of these regions was compared with the mechanical systole of the right auricle. The excitation was found to reach the auriculo-ventricular node and the right atrium at approximately the same time. Direct comparisons of the sino-auricular node with the surrounding parts indicated a more rapid spread of the excitation toward the venous and intercaval regions surrounding the node than toward the right atrium. In another series of experiments (2) estimation of the time relations of initial activity in the various parts before and after functional isolation was made.

The first of our experiments were criticised by Lewis, Meakins and White (3), who concluded from their own experimental data that the excitation arising within the sino-auricular node spreads out in all directions at approximately the same velocity. In a subsequent communication, Lewis and Rothschild (4) compare it to the spread of fluid poured upon an almost flat surface.

Lewis, Meakins and White paid considerable attention to what they designated as intrinsic and extrinsic effects obtained in direct leads from the heart to the galvanometer. The former is the expression of the electrical change of the active tissue (action current) at the electrode, while the latter is the influence which such changes in distant parts of the heart may exert upon the galvanometer by indifferent conduction through the cardiac tissue. They criticised our conclusions because of the belief that we had been unaware of this phenomenon. That there are such effects, no one who has worked much with this method could have failed to note, and we regard the statement of Lewis, Meakins and White that they have passed unobserved or ignored by other workers than themselves as gratuitous.[1] In direct comparisons in the supraventricular regions of the heart, so-called extrinsic movements are absent, as Lewis, Meakins and White have noted, in leads radial from the node, while in cross leads they can usually be excluded in forming an estimation of the passage of the excitation. In our experience, these doubtful curves are confined to contacts which receive the excitation simultaneously or nearly so, and this also seems to be the experience of Lewis, Meakins and White. Most of our determinations of the path of the negative wave have been made by the use of electrodes in pairs, applied to the heart surface and separated by a few millimeters or less. The curves obtained from electrodes of this type rarely show evidences of extrinsic effects, since any distant change of electrical condition propagated in a diffuse manner, is too attenuated in the small space between the contacts to affect the galvanometer appreciably. If experimental procedures are carried out, as in most of our experiments, to change rates of conduction by functional interruption of the paths, extrinsic effects are unchanged in their relations, while the change in the position of the intrinsic deflections denotes the change in the rate of propagation. When extrinsic deflections occur, as they do rarely in such curves, they are thus easily detected. It should be noted in this connection that Lewis and his co-workers, so far as we know, have not used the method of functional separation in studying the passage of the impulse from the

[1] It should be pointed out that their statement, "From the curves which they publish we think it highly improbable that accurate readings of the intrinsic deflections have been obtained," can refer only to figures 12, 13 and 14 in our paper, and of these curves only one shows a possible extrinsic deflection. If the measurement has been made from this instead of the intrinsic deflection, there would have been a difference of less than 0.0025 second, an amount which we regarded as within the limit of error of measurement.

sino-auricular node, and they thus fail to have, as a criterion for separation of extrinsic and intrinsic effects, that which is most valuable in this connection, namely, the change in the relation of intrinsic deflections and the absence of change in the extrinsic deflections as a result of interruption of the physiological path. It has been our experience, furthermore, that extrinsic effects occur more frequently and are more difficult to recognize in the application of one electrode of a pair to the heart and the other to the body tissue (as employed by us in our first experiments and subsequently by Lewis, Meakins and White and by Lewis and Rothschild), than when both electrodes are applied close together on the heart surface, as employed by us in our later work. It was this fact which caused us to abandon the former method in favor of the latter, in our later work. It was also this consideration which led to the development of the so-called differential electrode by Garten, in which the electrodes of each pair are in contact, so that they are connected by a path of less resistance than the heart muscle offers. Due to the necessity of a very loose galvanometer string in order to obtain satisfactory curves under such conditions and the possible errors entailed by a slowly moving, loose thread, we approximated this method by bringing the contacts close enough together to eliminate in large part the extrinsic influences, but with sufficient separation to obtain satisfactory deflections with the galvanometer in its usual range of sensitiveness.

It should finally be noted that extrinsic effects are much more common and confusing when contacts are placed on large complex masses of muscle, such as the mammalian ventricle, than on the simple muscle masses of the supraventricular regions. From our experience, in which attempts were early made by us to apply the electrical method to the mammalian ventricle in studying the course of the excitation wave and abandoned because of contradictory results and the recognition of the inherent difficulties, we are inclined to question seriously any conclusions drawn from the use of this method, in any form yet devised, in regard to the course of the excitation wave in the mammalian ventricle. The fact that the results obtained by Lewis and Rothschild (4) and Lewis (5) are contradictory to those obtained by Erfmann (6), we regard as an expression of the inapplicability of this method, in its present form at least, to the study of the excitation wave in the mammalian ventricle. With a number of muscle masses complexly related and coursing in different planes, many of which are probably in activity at the same time, and with the complex divisions and branching of the auriculoventricular conduction system, a condition results which makes it

extremely difficult to assume that the change recorded by the galvanometer is due to an action current at the point of contact of the electrodes.

The methods used by Lewis, Meakins and White, and by ourselves in our first publication on the subject of conduction from the sino-auricular node were similar. Direct comparisons of points in reference to the direction of movement of the galvanometer were used to map out the course of the wave, and measurements of velocity were made by comparing the movement of a galvanometer connected between some point on the heart and an indifferent point on the body surface with some more or less fixed standard of the cardiac cycle. Lewis, Meakins and White used the P wave of the axial electrocardiogram for a standard, while we employed the suspension curve of the right auricle. Examination of the published data shows also that the results obtained are in large part similar. Thus Lewis, Meakins and White find the highest velocity of conduction to the intercaval regions to be from 1121 to 1252 mm. per second, and the lowest to the right atrium, 859 mm. per second and to the superior vena cava 588 mm. per second. In the last instance our results differ, since we found a rapid propagation to this region. As stated by Lewis, Meakins and White, however, the distances in this case are short and measurements are subject to considerable error. These authors give only three determinations of the velocity of conduction to the auriculo-ventricular node, an average of 1101 mm. per second. In reference to comparisons between the time of arrival of the excitation at the auriculo-ventricular node and the right atrium in the same animal, Lewis, Meakins and White present no data, although in one experiment figures are given for this node and the right auricular appendage, the former preceding by approximately 0.004 second. These authors erroneously state our figure for node to node conduction as from 0.02 to 0.005 second. These figures were presented by us (p. 130) as the time, in five experiments, by which the occurrence of negativity in the a-v node preceded that in the right atrium near the sino-auricular node. The actual time of propagation between the nodes as determined by us from direct observation was placed at approximately 0.023 second[2]

[2] The values stated by us in a subsequent work obtained by the use of two galvanometers connected with modified differential electrodes and recording simultaneously were, Sa-Av., 0.023 second (28 experiments), Sa-Ra 0.020 (19 experiments. This method we regard as more accurate, since it avoids the uncertainty of comparison of the action current with curves in which the movements are not sharply defined (suspension curve of right auricle or P wave of the axial electrocardiogram), and which are probably subject to variation in their relative position.

which corresponds fairly closely with the figure of 0.03 second, obtained by Lewis, Meakins and White by an indirect method (p. 404).

While the fact that the wave of negativity courses more rapidly toward the venous side than to the auricular side of the sino-auricular node and actually reaches the auriculo-ventricular node at approximately the same time or only a very short interval after the much nearer region of the right atrium, points strongly to separate paths of conduction to these two regions, far more conclusive evidence, in our opinion, is obtained by partial or complete functional isolation of the paths concerned and the determination of the influence of such isolation on conduction. Experiments of this type, so far as we are aware, have been carried out only by ourselves (2). Determination of the time of conduction of the wave of negativity between the sino-auricular node, right atrium and the auricular and ventricular portions of the auriculo-ventricular node by contacts of the differential electrode type placed on these regions and connected to two galvanometers recorded simultaneously, proved that the position of the functional separation between the sino-auricular node and the surrounding tissue determined the influence exerted upon the rates of conduction between these different regions. Isolation of the right atrial border of the sino-auricular node did not increase the conduction time from this node to the auriculo-ventricular node, but did increase the conduction time to the right atrium (an average increase of 0.0115 second in eight experiments). Isolation of the superior, inferior and intercaval borders of the node likewise had no appreciable effect on conduction between the sino-auricular node and the auricle. The time of conduction from the sino-auricular node to the auriculo-ventricular node, was, however, increased by this procedure. The average increase of Sa-Av conduction time from isolation on these three sides was 0.0121 second. This degree of isolation also produced auriculo-ventricular rhythm in 82 per cent of all cases. Isolation on the auricular side alone failed to produce this rhythm in any of seventeen experiments. The conclusion from these experiments was that conduction from the sino-auricular node to the right atrium and to the auriculo-ventricular node occurs by separate paths. The former is a diffuse path represented by the contact of the right atrium with the node. The latter path is also either diffuse in nature, involving connections above, below and on the intercaval borders of the node, or there are numerous possible paths offering varying degrees of resistance to the passage of the impulse.

Further evidence for sino-ventricular conduction. We wish to offer in this communication further experimental evidence which, in the dog, we believe strongly supports the hypothesis of conduction from the sino-auricular node to the auriculo-ventricular node independently of the right atrium. This evidence was obtained from experiments in which the sino-auricular node was partially isolated by ligation and the animals allowed to recover. The details of this procedure are described in the preceding paper of this series. Two of the seven animals in the first series showed, after a period of auriculo-ventricular rhythm lasting for a day or more, sino-auricular rhythm with partial block between the supra-ventricular regions and the ventricles. These have been supplemented by two similar experiments carried out subsequently. Observations have thus been made on a total of four animals. When sino-auricular rhythm was reëstablished, conduction to the auricle was sufficiently normal to occur with every sinus impulse, but conduction to the ventricle was below normal and only approximately every other beat, arising within the sino-auricular node, was conducted to the ventricles. An example of this rhythm is given in figure 1. Only two explanations of this appear possible. If we assume that the excitation, arising within the sino-auricular node, passes to the auricles and thence to the auriculo-ventricular node and ventricles, the lesion producing the block must have been in this path and beyond the auricles. The auriculo-ventricular node and its ventricular connections were, however, far from the field of operative injury, and subsequent examination showed no evidence of extension of the injury to this region. If one assumes that conduction from the sino-auricular node to the auricles and to the auriculo-ventricular node occurs by separate paths, a lesion at or in the immediate neighborhood of the sino-auricular node might interfere with conduction through one path and not through the other. The conclusion that the auriculo-ventricular dissociation noted in these experiments was due to injury to the path of conduction between the sino-auricular and auriculo-ventricular nodes and that furthermore the path of conduction between the sino-auricular node and the auricles was not seriously interfered with, seems to us to be justified by the experimental facts, and to add further important evidence pointing to the presence of these two separate paths of conduction. We regard the condition in these experiments therefore as that of true sino-ventricular block.[3]

[3] The theoretical possibility of sino-ventricular block without disturbance of sino-auricular conduction has been discussed by us in a previous paper (7). A clinical case, probably to be interpreted as sino-ventricular block, has been described by Naish (8).

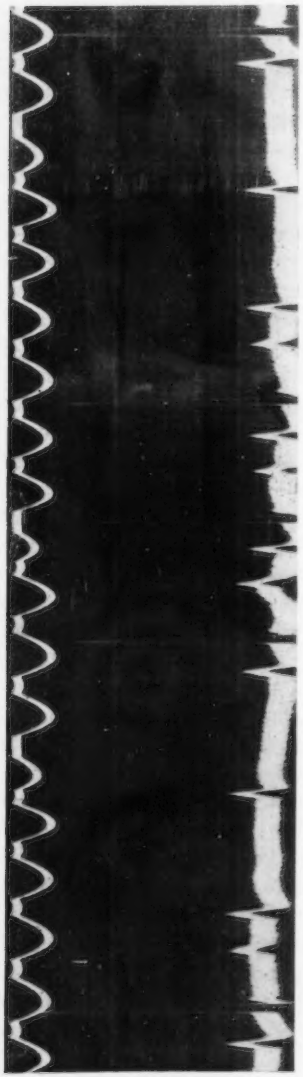

Fig. 1. Partial auriculo-ventricular dissociation consequent upon partial isolation of the sino-auricular node in the dog. Interpreted as partial sino-ventricular block.

SUMMARY

Previously published work, concerning the paths of conduction of the wave of negativity from the sino-auricular node to the right atrium and the auriculo-ventricular node in the mammalian heart, is reviewed.

New experimental evidence is presented which we believe tends to substantiate the view previously stated by us, namely, that conduction of the excitation from its origin in the sino-auricular node to the right atrium and auriculo-ventricular node respectively, occurs normally by separate paths. This evidence is the occurrence of auriculo-ventricular dissociation in dogs after partial isolation of the sino-auricular node.

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I. DETERMINATION OF PLASMA AND HEMOGLOBIN VOLUMES AFTER UNIT HEMORRHAGES UNDER CONTROLLED EXPERIMENTAL CONDITIONS

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Much experimental work has been done to study the effect of hemorrhage upon blood volume: observations on experimental animals and human patients after hemorrhage, with or without shock. Much of this work has been adequately reviewed by Bock (2) and we refer to his paper for the bibliography and a critical review of papers.

Our work here reported is somewhat different from that published by other investigators in that we made *simultaneous plasma volume and hemoglobin volume determinations* using methods previously described and controlled as to accuracy under the conditions of these experiments. The dye and hemoglobin method is described by Lee and Whipple (4). The carbon monoxide method is described by Arnold, Carrier, Smith and Whipple (1). The various limitations of these methods are reviewed with experimental control by Smith, Arnold and Whipple (5). This last paper pointed out the important fact that in the dog we record a plasma volume of approximately 5 per cent and a hemoglobin or red cell volume of approximately 4 per cent which indicates a total blood volume of 9 per cent. With the red cell hematocrit as 50 per cent we fall into error and usually calculate the total blood volume from the plasma figures as 10 per cent or from the carbon monoxide figures as 8 per cent making the error of assuming the ratio of cells to plasma remains constant in all parts of the blood stream as 50 per cent or the jugular red cell hematocrit value. We gave evidence that the axial stream in the arterioles and the flow through the capillaries can explain the difference and show where the extra 1 per cent of plasma is to be found. For a review and discussion of all this data we must refer the reader to the original paper (5).

It is becoming increasingly evident that the blood volume figures may be different in various animals of different species. We are familiar with the observations that the red and white cell counts may be very different in normal human beings, normal dogs and normal rabbits. Why should we assume that the circulating blood volume of these different animals should be a constant, whether calculated on the basis of body weight or body surface? We believe it wise to make no assumptions as to normal blood volume figures until accurate determinations have been made in man, dogs, rabbits, monkeys, etc. For accurate work it is obviously desirable to have direct determination figures both for the plasma volume and the hemoglobin volume.

Experimental observations. Dogs were used in all these experiments. These dogs were all healthy active adults. They had been long under observation in the laboratory and used for experiments of similar nature. They were familiar with laboratory and blood volume routine and were quite undisturbed by these procedures. They were receiving a liberal diet of hospital food scraps including meat, bones, potato, bread and milk, which has been shown to be a diet favorable for a rapid regeneration of red cells and hemoglobin after anemia (6). The dogs were fasted for 24 hours preceding the hemorrhage experiments to insure a waterclear plasma but water was in the cages at all times. These procedures caused the dogs no discomfort. Hemorrhage was produced by means of a hypodermic needle introduced into the jugular vein, the blood being aspirated into a flask containing isotonic oxalate solution. This hemorrhage blood was then measured and its hematocrit value determined.

The *tables* are of uniform type and give the necessary data. The dogs, unless note is made to the contrary, were not disturbed by a 25 per cent hemorrhage. *Figures* given in *bold type* indicate the *measured values;* for example, the CO method measures the red cell volume but the plasma volume by this method is *calculated.* In the same way the plasma volume only is *measured* by the dye method. The *true blood volume* is the sum of these two figures for plasma and red cell volume and is given in bold type. The *true red cell hematocrit* is calculated on the basis of the true blood volume and the red cell volume figures. This figure is a theoretical value which would be given if all the blood cells and blood plasma could be removed from a dog, thoroughly mixed, and a sample centrifugalized as usual.

We must not forget that about 8 to 10 per cent of body hemoglobin is *immobilized hemoglobin,* not truly circulating but taking part in

diluting the inhaled CO. This immobilized hemoglobin is contained in the muscles (myohematin) and bone marrow. This figure for immobilized hemoglobin is not allowed for in our tables as it probably

TABLE 1

Simultaneous blood volume determinations (plasma and red cells) after 2 bleedings of 25 and 20 per cent of true blood volume

Dog 21-56. White bull dog, female, adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
1/27	Hgb.	1461	**658**	803			55.0	116.1	52.2	63.9		46.5	12.6
	CO	1008	437	**571**			56.6	80.0	34.7	45.3	**1229**		
1/31	Hgb.	1335	**633**	702			52.6	106.0	50.2	55.8		46.0	12.6
	CO	979	439	**540**			55.2	77.7	34.8	42.9	**1173**		
2/1	5:00 p.m. Bled total 300 cc. Cells 160 cc. Plasma 140 cc. = 25 per cent of true blood volume												
2/2 2:00p.m.	Hgb.	1290	**706**	584	493		45.2	102.2	56.1	46.1		34.3	12.6
	CO	839	470	**369**		360	44.0	66.6	37.3	29.3	**1075**		
2/2	4:15 p.m. Bled total 215 cc. Cells 86 cc. Plasma 129 cc. = 20 per cent of true blood volume												
2/3 2:00p.m.	Hgb.	1275	**899**	376	577		29.5	101.1	71.3	29.8		25.3	12.6
	CO	960	655	**305**		269	31.8	76.2	52.0	24.2	**1204**		
2/4 2:30p.m.	Hgb.	1265	**896**	369			29.3	100.2	71.1	29.1		25.4	12.6
	CO	1013	707	**306**			30.2	80.4	56.1	24.3	**1202**		
2/7 2:30p.m.	Hgb.	1146	**786**	360			31.3	91.0	62.4	28.6		31.0	12.6
	CO	1033	681	**352**			34.1	82.0	54.0	28.0	**1138**		
2/11 3:00p.m.	Hgb.	1339	**864**	475			35.5	106.2	68.5	37.7		30.8	12.6
	CO	1023	639	**384**			37.5	81.2	50.8	30.4	**1248**		

does not change greatly during the periods of these experiments. Does the immobilized hemoglobin all become saturated with CO to the same extent as does the hemoglobin of the rapidly circulating red cells,—for

example the hemoglobin in the relatively inaccessible bone marrow? We hope to report experiments which will give data as to this immobilized hemoglobin and the fluctuation of muscle hemoglobin.

Unit hemorrhage followed in 18 to 24 hours by blood volume determinations. A considerable number of experiments was performed but only four are given in summary table 3. Some of these experiments lacked

TABLE 2

Simultaneous blood volume determinations (plasma and red cells) after 2 bleedings of 25 per cent and 17 per cent of true blood volume

Dog 21-64. Bull terrier, adult, female

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
2/1 3:30 p.m.	Hgb.	1255	**653**	602			48.0	129.3	67.3	62.0		34.6	9.7
	CO	752	406	**346**			46.0	77.5	41.9	35.6	**999**		
2/1	4:30 p.m. Bled 250 cc. Cells 112 cc. Plasma 138 cc. = 25 per cent of true blood volume												
2/2 12:00 p.m.	Hgb.	1023	**692**	331	515		32.4	106.5	72.0	34.5		24.9	9.6
	CO	696	468	**228**		220	32.7	72.5	48.8	23.7	**920**		
2/2	3:00 p.m. Bled 162 cc. Cells 53 cc. Plasma 109 cc. = 17 per cent of true blood volume												
2/3 3:00 p.m.	Hgb.	1017	**752**	265	583		26.1	101.7	75.2	26.5		21.6	10.0
	CO	776	568	**208**		163	26.8	77.6	56.8	20.8	**960**		

a detail here or there and in some the plasma volume alone or the hemoglobin volume alone were measured. All these data are in harmony and the examples cited are truly representative.

Table 1 is a good example of a characteristic experiment. The first hemorrhage was preceded by two control determinations. The determinations after the first hemorrhage were made 21 hours following the bleeding. The second bleeding was done just after these blood determinations on the same day and the blood volume determinations again made 22 hours after the second hemorrhage.

The 20-hour interval between each bleeding and blood volume measurement allowed complete compensation to take place and we note very little change in *total volume*. This means, of course, an obvious *increase* in plasma volume after each hemorrhage, more marked after the second hemorrhage. There is a slight increase in red cell volume

SUMMARY TABLE 3

Simple hemorrhage experiments with simultaneous blood plasma and red cell volume determinations. Compare measured and expected cell and plasma volumes

DOG NUMBER	WEIGHT	TIME	JUGULAR R. B. C. HEMATOCRIT	TRUE VOLUME COMBINED METHOD	PLASMA VOLUME HEMOGLOBIN METHOD	R. B. C. VOLUME CO METHOD	EXPECTED VOLUME		AMOUNT OF BLEEDING IN PERCENT OF BLOOD VOLUME
							Plasma	Cell	
	kgm.		*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
21–6	13.7	Before Hem.	48.0	1366	838	528			
		After 1 Hem.	37.7	1231	843	388	684	379	20
		After 2 Hem.	26.0	1167	872	295	663	273	23
21–56 Table 1	12.6	Before Hem.	55.2	1173	633	540			
		After 1 Hem.	44.0	1075	706	369	493	360	25
		After 2 Hem.	31.8	1204	899	305	577	269	20
21–64 Table 2	9.8	Before Hem.	46.0	999	653	346			
		After 1 Hem.	32.7	920	692	228	515	220	25
		After 2 Hem.	26.8	960	752	208	583	163	17
19–102	16.4	Before Hem.	60.8	1291	627	664			
		After 1 Hem.	43.0	1264	817	447	472	440	28
		After 2 Hem.	31.5	1292	944	348	592	282	29
Averages.....		Before Hem.	52.7	1207	682	519			
		After 1 Hem.	39.3	1122	764	358	541	349	24
		After 2 Hem.	28.8	1155	866	289	603	246	22

Hemorrhage in these experiments preceded the blood volume determinations by 18 to 22 hours.

after the second bleeding—compare red cell volume by the CO method, 305 cc. with the expected red cell volume 269 cc.

Table 2 again is representative of many experiments. The red cell hematocrit to begin with was a little subnormal but the experiment is similar in all respects to table 1. The second hemorrhage in both experiments was less than 25 per cent of the true blood volume. We

note again an increase in the true plasma volume 20 hours after each hemorrhage.

Summary table 3 gives four similar experiments including those of tables 1 and 2. It should be noted that there was no shock or prostra-

TABLE 4

Simultaneous blood volume determinations immediately after 2 bleedings of 18 per cent and 21 per cent of true blood volume

Dog 20–101. Airedale, young adult, female

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
4/7 3:00 p.m.	Hgb.	2288	**1096**	1192			52.1	114.4	54.8	59.6		44.7	20.0
	CO	1535	649	**886**			57.7	76.7	32.4	44.3	**1982**		
4/11	3:00–3:05 p.m. Bled 355 cc. Hemat. 50.0 per cent. Cells 178 cc. Plasma 177 cc. Blood Vol. det. 5 minutes later												
	Hgb.	1675	**872**	803	919		48.0	91.1	47.4	43.7		41.8	18.4
	CO	1385	755	**630**		690	45.5	75.4	41.1	34.3	**1502**		
4/12	3:00–3:05 p.m. Bled 325 cc. Hemat. 37.9 per cent. Cells 123 cc. Plasma 202 cc. Blood Vol. det. 5 minutes later												
	Hgb.	1492	**918**	574	993		38.5	78.2	48.1	30.1		34.2	19.1
	CO	1324	846	**478**		492	36.1	69.4	44.4	25.0	**1396**		
4/13	Hgb.	1585	**1081**	504			31.8	83.0	56.6	26.4		28.7	19.1
	CO	1400	965	**435**			31.1	73.4	50.6	22.8	**1516**		
4/15	Hgb.	1530	**1048**	482			31.5	81.4	55.7	25.7		33.2	18.8
	CO	1608	1085	**523**			32.5	85.5	57.7	27.8	**1571**		
4/20	Hgb.	1495	**954**	541			36.2	74.4	47.5	26.9		38.4	20.1
	CO	1545	949	**596**			38.6	77.0	47.3	29.7	**1550**		

tion following these hemorrhages in any of these experiments in table 3. A few other experiments presenting a mild grade of shock have not been included here but in such experiments we have noted no differences in reaction on the part of the plasma volume or hemoglobin volume.

The averages of these four experiments are of much interest and the close approximation of measured and expected hemoglobin values (cell volume CO method) is significant. These values after the first hemorrhage differ by (358 − 349 = 2.6 per cent) a percentage within the 5 per cent error of the methods. After the second hemorrhage we note an increase in measured over the expected values (289 − 246 = 17 per cent) of 17 per cent which may be in part explained by emergency formation of new hemoglobin but perhaps in part by mobilization of some of the "immobilized hemoglobin."

We wish to note that all the figures for *red cell volume CO method include this 8 to 10 per cent immobilized hemoglobin.* This amount of CO combining material is obviously not circulating red cells or hemoglobin but is probably reasonably constant and reduces by a fixed amount in each instance the figures given in the various tables. We shall take this subject up more in detail in a subsequent paper.

By contrast (table 3) we note a great increase in actual *plasma volume* after each hemorrhage but more after the second bleeding. For this reason the true total blood volume shows almost the same values before and after the bleedings. In the dog therefore we have evidence that an effort is made to keep a *constant total volume* of circulating blood, compensating for loss of red corpuscles by an increase in plasma. The average figures show an increase in plasma volume from 682 cc. control to 764 cc. after the first bleeding, to 866 cc. after the second bleeding. This last indicates a total increase of plasma volume of 27 per cent above control level. Many other similar observations are recorded elsewhere in connection with a study of hemoglobin regeneration after anemia (3).

Unit hemorrhages followed immediately by blood volume determinations. This group of experiments is quite different in that the hemorrhage is followed immediately by the blood volume determinations. The bleeding occupied 5 minutes. There was a short period of 5 minutes for adjustment. The simultaneous blood volumes were then determined within 15 to 20 minutes, the plasma volume preceding the CO method.

Table 4 is characteristic of many other experiments. It shows that the measured and expected values for plasma and red cells are quite close. There was no evidence of shock. Many other similar experiments were performed but need not be tabulated. In some similar experiments the blood plasma volumes only or hemoglobin volumes only were determined. The experimental results were in accord.

Table 5 was complicated by shock after the second bleeding but this did not disturb the usual reaction of plasma dilution as indicated by

TABLE 5

Simultaneous blood volume determinations immediately after 2 bleedings of 21 per cent and 25 per cent of true blood volume

Dog 21–93. Police dog, male adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME: Plasma	EXPECTED VOLUME: Cell	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO: Total blood volume	VOLUME PER KILO: Plasma volume	VOLUME PER KILO: R. B. C. volume	TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
3/8	Hgb.	1680	**783**	897			53.4	96.1	44.7	51.4		45.6	17.5
3:30 p.m.	CO	1281	622	**659**			51.4	73.2	35.6	37.6	**1442**		
3/9	12:55–1:03 p.m. Bled 315 cc. Hemat. 52.5 per cent. Cells 165 cc. Plasma 150 cc. Blood Vol. det. 5 minutes later.*												
	Hgb.	1236	**586**	650	633		52.6	73.5	34.8	38.7		48.1	16.8
	CO	1108	565	**543**		474	49.0	66.2	33.7	32.3	**1129**		
3/10	3:50–3:55 p.m. Bled 279 cc. Hemat. 41.6 per cent. Cells 116 cc. Plasma 163 cc. Blood Vol. det. 5 minutes later.*												
	Hgb.	1332	**764**	568	690		42.7	75.3	43.0	32.3		33.9	17.7
	CO	1170	778	**392**		413	33.5	66.2	44.0	22.2	**1156**		
3/11	Hgb.	1202	**830**	372			31.1	71.1	49.0	22.1		30.3	16.9
	CO	1240	879	**361**			29.1	73.4	52.0	21.4	**1191**		
3/14	Hgb.	1306	**944**	362			27.8	78.2	56.5	21.7		27.4	16.7
	CO	1267	911	**356**			28.1	75.8	54.5	21.3	**1300**		
3/18	Hgb.	1270	**921**	349			27.5	79.4	57.5	21.9		29.7	16.0
	CO	1379	989	**390**			28.3	86.2	61.8	24.4	**1311**		
4/6	Hgb.	1350	**803**	547			40.5	89.2	53.0	36.2		38.7	15.1
	CO	1295	790	**505**			39.0	85.8	52.3	33.5	**1308**		

* Dog shocked and CO method delayed 15 minutes (note red cell hematocrit): next day dog in poor condition; following days show recovery.

the CO hematocrit. The slow clinical recovery of this dog following the bleedings probably explains the very slow regeneration of red cells

on subsequent days, the level remaining almost stationary for one week.

Summary table 6 shows three similar experiments in which we may compare the average figures for plasma and red cell volumes as *determined* and as *calculated* after hemorrhage. We see these figures are very close to each other and variations do not exceed 4 per cent.

SUMMARY TABLE 6

Simple hemorrhage experiments with simultaneous blood plasma and red cell volume determinations immediately after bleedings. Compare measured and expected cell and plasma volumes

DOG NUMBER	WEIGHT	TIME	JUGULAR R.B.C. HEMATOCRIT	TRUE VOLUME COMBINED METHOD	PLASMA VOLUME HEMOGLOBIN VOLUME	R. B. C. VOLUME CO METHOD	EXPECTED VOLUME		AMOUNT OF BLEEDING IN PER CENT OF BLOOD VOLUME
							Plasma	Cell	
	kgm.		*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
17–154	23.7	Before Hem.	57.1	1936	**836**	**1100**			
		After 1 Hem.	54.0	1586	**711**	**875**	649	820	23
		After 2 Hem.	46.6	1542	**797**	**745**	680	650	28
21–93	17.5	Before Hem.	51.4	1442	**783**	**659**			
		After 1 Hem.	49.0	1129	**586**	**543**	633	474	21
		After 2 Hem.	33.5	1156	**764**	**392**	690	413	25
20–101	19.0	Before Hem.	57.7	1982	**1096**	**886**			
		After 1 Hem.	45.5	1502	**872**	**630**	919	690	18
		After 2 Hem.	36.1	1396	**918**	**478**	993	492	21
Averages.....		Before Hem.	55.4	1786	**905**	**881**			
		After 1 Hem.	49.5	1405	**723**	**682**	733	661	21
		After 2 Hem.	38.7	1364	**826**	**538**	787	518	24

A few experiments (table 7) were performed on a somewhat different plan. The dog was bled as usual but this procedure was followed by a prompt *intravenous infusion* of a gum acacia glucose solution. The blood volume determinations were made a few minutes later. The *measured* and the *calculated* amounts of hemoglobin and red cells before and after this procedure are practically identical. There is a slight *increase* in the measured plasma volume over the calculated values indicating a slight tendency to make up the lost plasma during the hemorrhage period. Apparently this bleeding and the infusion of this solution does not in any way disturb the circulating blood volume.

TABLE 7

Simultaneous blood volumes done immediately after unit bleeding and infusion with glucose-acacia solution

Dog 21–5. Fox terrier, male adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
5/23 11:00 a.m.	Hgb.	708	**349**	359			50.6	109.5	54.1	55.5		40.1	6.46
	CO	449	216	**233**			52.0	69.4	33.3	36.1	**582**		
5/24	10:55 a.m. Bled 232 cc. Cells 107 cc. Plasma 125 cc. 11:10–11:20 a.m. Injected 200 cc. of 6 per cent glucose and 3 per cent gum acacia solution at 36°C. Blood volume determined 5 minutes later												
	Hgb.	634	**475**	159	424		25.0	103.2	77.4	25.8		18.8	6.14
	CO	397	287	**110**		118	27.7	64.8	46.8	18.0	**585**		

Dog 21–111. Fox terrier, male, adult

5/23 1:00 p.m.	Hgb.	680	**344**	336			49.4	119.6	60.5	59.1		36.4	5.68
	CO	447	250	**197**			44.2	78.7	43.9	34.8	**541**		
5/24	1:25 p.m. Bled 230 cc. Cells 93 cc. Plasma 137 cc. 1:29 p.m. Injected 200 cc. of 6 per cent glucose and 3 per cent gum acacia solution at 36°C. 1:38 p.m. Blood volume determined												
	Hgb.	588	**473**	115	407		19.5	107.9	86.8	21.1		17.4	5.45
	CO	429	329	**100**		96	23.4	78.6	60.2	18.4	**573**		

CONCLUSION

Simultaneous determinations of plasma volume and hemoglobin red cell volume can be rapidly made in dogs without clinical disturbance.

Such determinations done *immediately* after a unit hemorrhage show a striking similarity between the *measured* and the *calculated* values. This indicates accuracy of measurement and should increase our confidence in the accuracy of these methods under the conditions of these experiments.

Determinations done immediately after a unit hemorrhage and intravenous infusion of a glucose-acacia solution show close approximation of *measured* values and *calculated* values.

Determinations done 18 to 24 hours after a unit hemorrhage show the expected values in hemoglobin or red cell values but a considerable *increase* in measured plasma volume. The normal dog after hemorrhage *increases its plasma volume* to make up almost completely for the loss of red cells. This plasma volume decreases to the normal level as the red cells are regenerated to normal.

We recognize that approximately 8 to 10 per cent of the hemoglobin measured is "immobilized hemoglobin" held in the muscles and bone marrow. This is not allowed for in our own figures and will be discussed in a subsequent paper. It is obviously not true circulating red cells or hemoglobin but is capable of diluting the inhaled CO.

Finally these experiments confirm our opinion concerning the total blood volume in the dog. In our opinion the blood volume is made up of plasma (approximately 5 per cent of body weight) and red cells (approximately 4 per cent of body weight) although the jugular vein hematocrit reads 50 per cent, an equal division between plasma and cells. The details of this argument are given elsewhere (5).

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II. SIMULTANEOUS DETERMINATIONS OF PLASMA AND HEMOGLOBIN VOLUMES

Influence of Fluids by Mouth and Vigorous Exercise

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In the preceding paper there is evidence that the dog tends to maintain a rather uniform total blood volume. When there is a considerable loss of red cells we note a compensating increase in plasma volume which again decreases as new red cells are regenerated. The increase in plasma volume may not make up the loss in red cells and return the total volume to 100 per cent but the plasma increase may amount to 25 per cent or more. How far this increase in plasma volume will continue as increasing amounts of red cells are lost we cannot say. Our experience in this respect is different from the observations of Bock (1) and others to the effect that in man the plasma volume is a constant in spite of loss of red cells. It will be of interest to supplement these casual observations by more complete studies of human cases in which venesection is to be done as a remedial measure (heart disease, eclampsia, etc.). In a large series of human cases the total blood volume before and after a measured bleeding should be directly measured.

We thought it proper to determine the blood volume values during certain periods of fluid intake and vigorous exercise. To understand the physiological fluctuations in blood volume obviously we must know what physiological activities may influence plasma or total hemoglobin volumes. It may be stated that liberal water intake by stomach does increase distinctly the plasma volume. On the contrary a concentrated sugar solution has a negative effect.

One is surprised at first to learn that vigorous but brief exercise does not modify the total plasma or hemoglobin volumes. Dogs exercised at a run for 20 minutes may lose 12 ounces or more in weight and this is a fluid loss but the blood plasma volume is held at its normal level. There is no increase in the red cell hematocrit after exercise to correspond

with the familiar observations that the red count may rise after exercise (man).

Experimental observations. These experiments were done during the same period as the experiments in the preceding paper. General methods and experimental details are described above. The same standard dogs were used and these procedures caused the animals no discomfort. These experiments are uniform and the averages of many such experiments are convincing. Many incomplete experiments are not included in the tables but the experimental data are all in accord.

Table 8 gives in detail four representative experiments. The last experiment was complicated by vomiting but active diuresis was established and we have no evidence that vomiting under these conditions modifies the blood plasma volume increase. The first experiment shows a maximal increase of 400 cc. in plasma volume. Animals vary in their reaction to water drinking but in reviewing individual variations we must keep in mind the 3 to 5 per cent error of this method.

Table 9 gives a summary of eight complete experiments with water ingestion. Some of these experiments are complicated by vomiting but the reaction is not modified. The average of all experiments shows an increase in blood plasma volume from 964 cc. to 1143 cc. or 16 per cent increase. The red cell or hemoglobin volume remains practically unchanged. The average figures show a slight drop in red cell volume from 863 cc. to 846 cc. The jugular hematocrit shows a distinct fall from 53.3 per cent to 48.7 per cent.

Table 10 gives four complete experiments dealing with the ingestion of sugar solutions. The sugar solutions were usually given all at one time but in one experiment (dog 19–128) in divided doses. Glucose and cane sugar were used individually and combined and a variety of concentrations employed. The net result shows constant figures for the blood plasma and hemoglobin volumes. Vomiting does not appear to modify the reaction.

Table 11 gives a summary of seven complete experiments with ingestion of sugar solutions. The averages for all experiments are of particular interest and serve as control for the water experiments. The figures are practically identical before and after sugar ingestion.

Table 12 gives four representative complete experiments dealing with vigorous brief exercise. The dogs were exercised at a brisk run for a period indicated either on leash or behind an automobile. The dogs were panting and ready to lie down but not exhausted at the end of such periods. These dogs in the majority of instances were not ac-

TABLE 8

Water given by stomach tube. Blood and plasma volume increased

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R.B.C. HEMATOCRIT	VOLUME PER KILO: Total blood volume	VOLUME PER KILO: Plasma volume	VOLUME PER KILO: R. B. C. volume	TRUE VOLUME COMBINED METHOD	TRUE R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
Dog no. 19–128. Hound, adult, male											
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kgm.*
4/7	Hgb.	2340	**1080**	1260	53.9	82.2	37.9	44.3			
	CO	2150	968	**1182**	55.0	75.5	34.0	41.5	**2262**	**52.3**	28.5
4/8	9:00 a.m. 1000 cc. water; 11:00 a.m. 500 cc. water										
	10:00 a.m. 1000 cc. water; 11:30 a.m. 500 cc. water										
	10:30 a.m. 1000 cc. water; 12:15 p.m. Blood volume estimated										
	Hgb.	2867	**1490**	1377	48.0	102.7	53.4	49.3			
	CO	2252	1126	**1126**	50.0	80.7	40.3	40.4	**2616**	**43.0**	27.9
Dog no. 21–11. Collie, adult, female											
12/1	Hgb.	1935	**1054**	881	45.5	60.5	33.0	27.5			
	CO	1480	740	**740**	50.0	46.3	23.2	23.1	**1794**	**41.2**	32.0
12/2	9:15 a.m. 1000 cc. water; 11:00 a.m. 500 cc. water										
	9:45 a.m. 500 cc. water; 12:00 m. Blood volume estimated										
	10:30 a.m. 500 cc. water										
	Hgb.	2140	**1155**	985	46.0	67.5	36.5	31.0			
	CO	1572	839	**733**	46.6	49.6	26.5	23.1	**1888**	**38.8**	31.7
Dog no. 21–6. Hound, young adult, male											
12/3	Hgb.	1547	**859**	688	44.5	98.5	54.7	43.8			
	CO	1132	628	**504**	44.5	72.2	40.1	32.1	**1363**	**36.9**	15.7
12/4	8:30 a.m. 750 cc. water; 11:45 a.m. 750 cc. water										
	9:15 a.m. 500 cc. water; 12:15 p.m. Blood volume estimated										
	10:00 a.m. 750 cc. water										
12/4	Hgb.	1475	**959**	516	35.0	95.8	62.3	33.5			
	CO	1220	766	**454**	37.2	79.2	49.7	29.5	**1413**	**32.1**	15.4

TABLE 8—*Concluded*

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	TRUE R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
						Total blood volume	Plasma volume	R. B. C. volume			
Dog no. 19–128. Hound, adult, male											
1930		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kgm.*
3/24	Hgb.	2475	**1090**	1385	56.0	90.0	39.6	50.4			
	CO	1885	825	**1060**	56.3	68.6	30.0	38.6	**2150**	**49.3**	27.5
3/25	9:20 a.m. 1000 cc. water 10:50 a.m. 1000 cc. water 9:50 a.m. 1000 cc. water 11:20 a.m. 1000 cc. water 10:20 a.m. 1000 cc. water Dog vomited. Diuresis well established. 12:00 m. Blood volume estimated										
3/25	Hgb.	2440	**1187**	1253	51.4	89.0	43.3	45.7			
	CO	2080	999	**1081**	52.0	76.0	36.5	39.5	**2268**	**47.6**	27.4

SUMMARY TABLE 9

Increase in blood plasma volume due to water by mouth

DOG NUMBER	WEIGHT	BEFORE GIVING WATER				AFTER GIVING WATER				WATER AMOUNT GIVEN
		Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	
Uncomplicated by vomiting										
	kilos	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*liters*
18–4	18.2	55.2	**865**	**722**	1587	54.1	**882**	**835**	1717	5.0
19–128	28.5	54.5	**1080**	**1182**	2262	49.0	**1490**	**1126**	2616	4.0
21–11	32.0	47.8	**1054**	**740**	1794	46.3	**1155**	**733**	1888	2.5
21–6	15.7	44.5	**859**	**504**	1363	36.1	**959**	**454**	1413	2.8
17–154	25.3	59.8	**930**	**1130**	2060	54.7	**1186**	**1052**	2238	2.5
Average.....	23.9	52.3	**957**	**855**	1812	48.0	**1134**	**840**	1974	
Complicated by vomiting but diuresis well established										
19–128	28.2	56.5	**1065**	**1029**	2094	51.7	**1410**	**924**	2334	5.0
19–128	27.5	56.2	**1090**	**1060**	2150	51.7	**1187**	**1081**	2268	5.0
21–56	14.8	50.6	**762**	**525**	1287	45.3	**859**	**552**	1411	2.5
Average.....	23.5	54.4	**972**	**871**	1843	49.5	**1152**	**852**	2004	
Average all experiments.....	23.7	53.3	**964**	**863**	1827	48.7	**1143**	**846**	1989	

TABLE 10

Sugar solution given by stomach tube. Blood plasma volume unchanged

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO: Total blood volume	VOLUME PER KILO: Plasma volume	VOLUME PER KILO: R. B. C. volume	TRUE VOLUME COMBINED METHOD	R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
	Dog no. 19–128. Hound, adult, male										
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
8/5	Dye	2610	**1262**	1348	52.6	101.6	49.1	52.5			
	CO	2093	936	**1157**	55.3	81.5	36.5	45.0	**2419**	**47.8**	25.7
8/6	10:15 a.m. 500 gm. of sucrose in 350 cc. warm water 1:15 p.m. Blood volume dye method 2:15 p.m. Blood volume CO method										
8/6	Dye	2500	**1244**	1256	50.3	99.3	49.4	49.9			
	CO	2078	946	**1132**	54.5	82.4	37.5	44.9	**2376**	**47.7**	25.2
	Dog no. 19–128. Hound, adult, male										
1921											
5/19	Hgb.	2469	**990**	1479	59.9	96.6	38.7	57.9			
	CO	1967	744	**1223**	62.2	77.1	29.1	48.0	**2213**	**55.4**	25.5
5/13	9:30 a.m. 75 gm. glucose + 25 gm. sucrose in 800 cc. warm water 10:30 a.m. 75 gm. glucose + 25 gm. sucrose in 800 cc. warm water 11:30 a.m. 75 gm. glucose + 25 gm. sucrose in 800 cc. warm water 12:30 p.m. Blood volume estimated										
5/13	Hgb.	2427	**995**	1432	59.0	95.4	39.1	56.3			
	CO	2026	810	**1216**	60.0	80.0	32.0	48.0	**2211**	**55.0**	25.4
	Dog no. 18–60. Bull, old male										
1920											
6/22	Hgb.	2071	**976**	1095	52.9	86.0	40.5	45.5			
	CO	1983	972	**1011**	51.0	82.4	40.4	42.0	**1987**	**51.0**	24.1
6/23	10:30 a.m. 890 gm. of sucrose in 980 cc. of warm water 12:30 p.m. Dog vomited 200 cc. 2:00 p.m. Blood volume estimated										
6/23	Hgb.	2070	**915**	1155	55.8	87.8	38.8	49.0			
	CO	1866	802	**1064**	57.0	79.0	34.0	45.0	**1979**	**53.9**	23.6

TABLE 10—*Concluded*

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
						Total blood volume	Plasma volume	R. B. C. volume			
Dog no. 17–160. Hound, adult, male											
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
6/24	Hgb.	2338	**1159**	1179	50.4	96.7	48.0	48.7			
	CO	1750	818	**932**	53.2	72.4	33.9	38.5	**2091**	**44.6**	24.2
6/25	11:00 a.m. 1170 gm. of sucrose in 1300 cc. warm water 12:00 m. Dog vomited 400 cc. 2:00 p.m. Blood volume estimated										
6/25	Hgb.	2373	**1148**	1225	51.6	100.0	48.4	51.6			
	CO	1750	825	**925**	52.8	73.8	34.8	39.0	**2073**	**44.6**	23.7

SUMMARY TABLE 11

Blood plasma volume unchanged by ingestion of concentrated solution of sugar

DOG NUMBER	WEIGHT	BEFORE GIVING SUGAR				AFTER GIVING SUGAR				SUGAR SOLUTION	
		Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	Sugar	Water
Uncomplicated by vomiting											
	kilos	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*grams*	*cc.*
19–128	27.4	57.5	**1134**	**1152**	2286	59.6	**1052**	**1182**	2234	500	300
19–128	25.7	53.9	**1262**	**1157**	2419	52.4	**1244**	**1132**	2376	500	350
21–6	10.6	37.8	**763**	**378**	1141	36.7	**747**	**355**	1102	50	150
19–128	25.5	61.0	**990**	**1223**	2213	59.5	**995**	**1216**	2211	300	240
Average.....	22.3	52.5	**1037**	**977**	2014	52.0	**1009**	**971**	1980		
Complicated by vomiting											
18–60	24.1	52.0	**976**	**1011**	1987	56.4	**915**	**1064**	1979	890	980
17–160	24.2	51.8	**1159**	**932**	2091	52.2	**1148**	**925**	2073	1170	1300
19–102	18.3	58.6	**798**	**713**	1511	58.8	**765**	**762**	1527	400	300
Average.....	22.2	54.1	**977**	**885**	1863	55.8	**942**	**917**	1859		
Average all experiments.....	22.2	53.3	**1007**	**931**	1938	53.9	**975**	**944**	1919		

TABLE 12

Simultaneous determinations of blood plasma and red cell volume after vigorous exercise

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	TRUE R. B. C. HEMATOCRIT COMBINED METHODS	WEIGHT
						Total blood volume	Plasma volume	R. B. C. volume			
		Dog no. 18–4. Sheep dog, adult, female									
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kgm.*
4/19	Hgb.	2100	**1080**	1020	48.6	114	58.5	55.5			
	CO	1710	855	**855**	50.0	93	46.5	46.5	**1935**	**44.2**	18.4
4/20	9:30 a.m.–10:00 a.m. Dog exercised at a run on leash; well exercised and panting though not very tired										
	10:00 a.m.–10:20 a.m. Blood volume estimated										
4/20	Hgb.	1815	**925**	890	49.0	102.5	52.3	50.2			
	CO	1754	860	**894**	51.0	99.1	48.6	50.5	**1819**	**49.1**	17.7
		Dog no. 20–80. Hound, young adult, male									
4/22	Hgb.	2478	**1243**	1235	49.9	133.7	67.0	66.7			
	CO	1376	681	**695**	50.5	74.4	36.8	37.6	**1938**	**35.9**	18.5
4/23	11:15 a.m.–11:45 a.m. Dog exercised at run behind automobile; dog panting but not tired										
	11:45 a.m.–12:15 p.m. Blood volume estimated										
4/23	Hgb.	2060	**1084**	976	47.4	111.2	58.6	52.6			
	CO	1430	736	**694**	48.5	77.3	39.8	37.5	**1778**	**39.0**	18.5
		Dog no. 19–128. Hound, adult, male									
4/29	Hgb.	2561	**1136**	1425	55.6	92.9	41.3	51.6			
4/28	CO	1884	731	**1153**	61.2	68.3	26.5	41.8	**2289**	**50.4**	27.6
4/30	11:00 a.m.–11:12 a.m. Dog exercised at a run on leash										
	11:15 a.m. Blood volume estimated										
4/30	Hgb.	2550	**1190**	1360	53.4	94.8	44.2	50.6			
	CO	1943	894	**1049**	54.0	72.3	33.3	39.0	**2239**	**46.9**	26.9
		Dog no. 20–101. Airedale, young adult, male									
1921											
1/13	Hgb.	1936	**968**	968	50.0	90.3	45.1	45.2			
	CO	1794	884	**910**	50.7	83.6	41.2	42.4	**1878**	**48.5**	21.46
1/4	2:00 p.m. Dog exercised at a run behind car, rate 12 miles per hour for 3 miles										
	2:15 p.m. Blood volume estimated										
1/4	Hgb.	2101	**1006**	1095	52.1	98.0	47.0	51.0			
	CO	1705	817	**888**	52.1	79.5	38.1	41.4	**1894**	**46.9**	21.46

customed to vigorous exercise and many of them were rather fat. We have observed no difference in these experiments between the active lean dogs and the fat inactive ones although we have pointed out the fact that the active dogs possess a higher plasma volume per kilo body weight (2).

SUMMARY TABLE 13

No uniform change in blood plasma or red cell volume immediately following vigorous exercise

DOG NUMBER	WEIGHT	BEFORE EXERCISE					AFTER EXERCISE				
		Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	R. B. C. hematocrit combined methods	Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	R. B. C. hematocrit combined methods
	kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*
18–4	18.4	49.3	**1080**	**855**	1935	44.2	50.0	**925**	**894**	1819	49.1
20–80	18.5	50.2	**1243**	**695**	1938	35.9	48.0	**1084**	**694**	1778	39.0
19–128	27.6	58.4	**1136**	**1153**	2289	50.4	53.7	**1190**	**1049**	2239	46.9
19–128	27.1	59.7	**1147**	**1193**	2340	51.0	61.0	**882**	**1250**	2132	58.6
19–128	26.1	47.2	**1117**	**1020**	2137	47.8	45.1	**986**	**990**	1976	50.1
17–154	23.2	57.6	**995**	**1060**	2055	51.6	59.3	**1049**	**1102**	2151	51.3
19–102	16.8	50.8	**875**	**663**	1538	43.1	43.4	**865**	**644**	1509	42.6
21–11	31.8	46.7	**1075**	**665**	1740	38.2	47.7	**980**	**692**	1672	41.4
19–102	17.2	54.8	**708**	**610**	1318	46.3	51.0	**793**	**574**	1367	42.0
20–101	22.2	58.3	**906**	**938**	1838	50.7	55.0	**985**	**804**	1789	44.9
19–102	18.1	53.7	**772**	**661**	1433	46.1	50.4	**880**	**605**	1485	40.7
20–101	21.4	50.3	**968**	**910**	1878	48.5	52.1	**1006**	**888**	1894	46.9
21–6	15.2	54.0	**737**	**568**	1305	43.5	51.2	**712**	**556**	1268	43.9
Average..	21.8	53.1	**982**	**845**	1827	45.9	49.8	**950**	**829**	1779	46.6

Table 13 gives a summary of thirteen complete exercise experiments. The exercise periods varied from 10 minutes to 30 minutes of brisk running. The majority of experiments were of 12 to 15 minutes duration and averaged about 2 to 3 miles distance. We note a slight decrease in plasma volume in the total average figures but this 3 per cent decrease is too small to be significant. The hemoglobin or red cell volume likewise shows a slight decrease in the total averages.

Discussion. Copious *water ingestion* will obviously increase the blood plasma volume,—in our experiments the average increase is 16 per cent with variations between 10 and 40 per cent. In these

experiments the water was given in large amounts and a very active diuresis was established. It may be safe to assume that these increases in plasma volume are maximal but it is obvious that small amounts of water by stomach may have some influence on the plasma volume. It will be interesting to determine how long this increase in blood plasma volume persists—whether, for example, it lasts only during the period of very active diuresis. One suspects that this increase in plasma volume may be in part responsible for the great secretory activity of the kidney, but there are many other factors which must be weighed in an analysis of this problem.

Sugar solutions given by mouth exert no influence on the blood plasma volume. Concentrations of varying degree were used with similar result. We suspected the possibility that such solutions might cause a decrease in plasma volume due to intestinal fluid secretion. We also considered the possibility that the rise in blood sugar might be sufficient to call fluid from the tissues into the blood stream. The diffusion of the sugar solution in the body is evidently sufficiently rapid to prevent any such disturbance in the equilibrium of the fluids in the blood plasma. If any further method control is needed these sugar experiments furnish the data to contrast with the water drinking experiments.

The *exercise experiments* are of much interest but supply only a part of the data needed for a complete understanding of the physiology of the blood volume in exercise. These experiments are concerned with vigorous exercise periods of 10 to 30 minutes but we have no information concerning long exercise periods with or without clinical fatigue or exhaustion. When one considers the great transformation in the circulating system which must take place immediately during sudden transition from resting to active exercise or even to active digestion, it is a surprise that the blood volume is practically a constant. The blood volume remains a constant when there is a great change in the entire stream bed—for example, the active blood supply of a functioning muscle as contrasted with the resting organ. The average of thirteen complete experiments (table 13) shows a plasma volume of 982 cc. before and 950 cc. after vigorous exercise, a decrease of 3 per cent. The individual fluctuations may be almost 10 per cent. We can place no emphasis on this 3 per cent decrease in plasma volume after short exercise but if experiments of longer duration show continued plasma decrease it would be significant. There are many other points which might be discussed in connection with these experiments but we wish to reserve this discussion for a subsequent paper.

SUMMARY

Copious water ingestion will cause a distinct rise in total blood plasma volume. Our experiments show an average increase of 16 per cent.

Sugar solutions by mouth do not influence the blood plasma which remains a constant.

Short periods of vigorous exercise do not modify the blood plasma volume to a convincing degree. There are individual fluctuations but the average figures show a 3 per cent decrease after exercise. Whether this has any significance must be determined by prolonged exercise experiments.

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THE MECHANISM OF THE REGULATION OF INTRA-ABDOMINAL PRESSURE

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Much has been written about the conduct of the abdominal musculature under varying conditions. Intra-abdominal pressure under approximately normal circumstances has been studied on the one hand, and on the other we have a somewhat disconnected series of reports on the activity of the abdominal musculature. It is the object of this paper to attempt to bring the two into relationship, studying as many concomitant factors as may serve to make a larger or clearer picture of the conduct of this physiological mechanism as a whole.

The early work on intra-abdominal pressure consisted mainly in a study of its relation to inspiratory and expiratory changes. Marey (1) in 1863 and Paul Bert (2) in 1870 showed the relationship of the type of respiration to intra-abdominal pressure. Schatz (3) in 1873 and Schroeder (5) in 1886 suggested from gynecological evidence that there is an adjustment of the abdominal muscles to meet distention by the pregnant uterus. Weisker (7) (1888) pointed out that pressures in the hollow viscera may vary considerably and are independent of intra-abdominal pressure. Hasse (6) in 1890 noted that intra-abdominal pressure increased with the descent of the diaphragm in inspiration. He further considered that a fluctuating positive abdominal pressure is the result of simultaneous contraction of the diaphragm and the abdominal muscles in inspiration. Heinricius (8) (1890) found in cats and guinea pigs than an intra-abdominal pressure of 27 to 46 cm. of water was fatal, owing to prevention of respiration by interference with thoracic expansion.

Weitz (9) in 1909 on inserting a trocar in abdominal paracentesis for the withdrawal of ascitic fluid found that the fluid was under positive pressure, due to a combination of the forces of hydrostatic pressure and tension of the abdominal wall. Emerson (10) (1911) in etherized and decerebrated cats, dogs and rabbits found that contraction of the

diaphragm is the chief, if not the only factor in the normal rise in pressure during quiet inspiration. He concludes that excessive pressure artificially produced within the peritoneal cavity causes death from cardiac failure before the obstruction to respiration has developed a marked asphyxia. Burton-Opitz (11) (1914) showed that the amount of both carotid and jugular blood flow per second is raised by increasing intra-abdominal pressure. Pike and Coombs (12) (1917) showed a reflex lengthening of the rectus abdominis muscle on distention of the abdomen with saline. The reflex nature of its activity was demonstrated by the fact that after section of the appropriate dorsal roots of the spinal nerves, there was no further change in the length of the muscle.

Since Sherrington (14) has enunciated the doctrine of a reflex muscular tonus in which the muscle fiber lengthens without increase in tension, much work has been done illustrative of the activity of this function in smooth muscle viscera, such as the bladder and stomach (13). A review of the literature on this phase of the work would be superfluous here. It is my intention to confine the discussion to the conduct of a certain group of skeletal muscles.

If, as Sherrington has pointed out, there is a reflex ability of certain muscle fibers to lengthen without change in tension, we should take as an index of this tonicity in the abdominal musculature the ability to show different lengths with relatively constant tension, or, looking at it conversely, to demonstrate within the abdominal cavity a fairly constant pressure with varying increments of volume.

It is the consensus of opinion of the previous experimenters that under normal conditions, with the viscera entirely filling the cavity, there is a slight positive pressure in the abdomen. An increase in the volume of the abdominal contents should, by the accompanying variations in pressure, throw some light on the mechanism of the activity of the abdominal wall. With this in mind, a series of experiments has been carried on.

Experimental work. Cats were used under light and deep anesthesia and after decerebration. A cannula was introduced into the abdominal cavity and connected by a 3-way stopcock with a manometer and a burette filled with Ringer's solution kept at a temperature of 38°C. In many cases costal respiration was recorded throughout the experiment. Blood pressure also was taken on a large number of cats. The warmed Ringer's solution was admitted to the abdominal cavity at the rate of 10 cc. a minute. With each increment of fluid the pressure was read from the manometer and plotted against the volume injected.

A control curve was thus obtained which in about seventy-five cases was found to be typical. There was a slow increase of pressure in proportion to volume until a certain critical point was reached, from which pressure rose much more rapidly in proportion to volume. At this point also costal respiration increased very greatly in depth to effect compensation for the lack of adequate abdominal respiration. Arterial blood pressure remained unaffected.

In many cases the experiments were continued until a failure of respiration was threatened, which was likely to occur when the pressure had reached 250 to 400 mm. of Ringer's fluid, although in pregnant cats it has been observed to rise to more than 1000 mm. before respiration became dyspneic.

After this control curve was obtained, the fluid was withdrawn from the abdominal cavity, and about half an hour was allowed to elapse before the next part of the experiment, in which the procedure was varied. Since we have the activity of two sets of mutually antagonistic muscles to consider, a lesion of the nervous mechanism of first one set and then of the other, was made.

1. In some cases the dorsal roots of the spinal nerves in the lower thoracic and lumbar regions were cut in order to determine the effect of abolition of the sensory mechanism of the abdominal muscles.

2. In other cases the spinal cord was transected in the mid-thoracic region, causing paralysis of the abdominal musculature.

3. In other cases the three branches of each phrenic nerve were divided in the neck, causing paralysis of the diaphragm.

4. In order to eliminate all reflex activity, curare was injected intravenously in a number of cases, with maintenance of artificial respiration.

After each of these operative procedures the abdominal cavity was filled with saline and the pressure was plotted against the volume as before. In a purely elastic fundus the curve shows pressure increasing in direct proportion to volume. The smaller the increase in pressure in proportion to volume, the greater, then, would be the change in the length of a muscle fiber without change in tension. We would therefore expect the shape of the curve obtained to be some indication of the activity of the muscle fiber apart from the purely elastic quality shown in the dead animal.

After such a curve was obtained the fluid was again withdrawn, and in many cases the cat was killed by closing the trachea. Two hours and four hours after death curves were taken in a similar manner, in

which the passing over of the musculature into the state of elasticity was clearly shown.

Following are protocols of typical experiments on each phase of the question.

January 26, 1920. Male cat, weight 3100 grams.
Ether, laminectomy from the mid-thoracic region through the lumbar part of the spinal cord.
Cannula introduced into abdominal cavity and connected with the recording apparatus.
10:30 a.m. Introduction of Ringer's solution in amounts of 10 cc. at each injection, begun. Curve indicates slow rise in pressure until about 600 cc. of fluid has been injected, then shows a more rapid rise in pressure.
11:50 a.m. Respiration labored. Pressure 250 mm. of Ringer's solution with 800 cc. fluid in abdomen. Injection stopped.
11:55 a.m. Fluid removed from abdominal cavity. Respiratory rate increases and abdominal respiration returns.
12:20 p.m. Spinal cord transected and removed, mid-thoracic through lumbar.
12:40 p.m. Ringer's solution again injected and curve plotted. There is a more rapid rise in pressure than in the normal curve.
2:10 p.m. Respiration labored, no more fluid run in. The pressure is 260 mm. of Ringer's fluid, while the volume injected is 700 cc.
2:15 p.m. Fluid removed, respiratory rate increases.
2:20 p.m. Cat killed by occlusion of trachea.
4:00 p.m. Fluid run in and curve plotted; 600 cc. of Ringer's solution gives a pressure of 260 mm. fluid.

It will be seen from the curves obtained that in paralysis of the intercostal mechanism the pressure in the abdomen increases somewhat in proportion to the volume (chart 1).

March 23, 1920. Female cat, weight 3375 grams. Ether, tracheotomy. Cannula in abdomen, connected with the recording apparatus.
10:00–11:10 a.m. Curve of intra-abdominal pressure taken. When the pressure reaches 230 mm. of Ringer's solution, on injection of 700 cc. of fluid, respiration becomes labored. The fluid is then withdrawn from the abdominal cavity, and respiration regains its former rate.
11:20–11:30 a.m. Phrenics removed, all three branches on each side. Diaphragm paralyzed.
11:40–12:40 p.m. 600 cc. fluid injected, pressure 250 mm. of Ringer's solution. Fluid removed.
12:50 p.m. Cat killed by occlusion of trachea.
3:00–4:00 p.m. 600 cc. fluid injected, pressure 300 mm. of Ringer's fluid.

Paralysis of the diaphragm, to a somewhat greater degree than paralysis of the muscles of the abdominal wall such as the rectus abdominis,

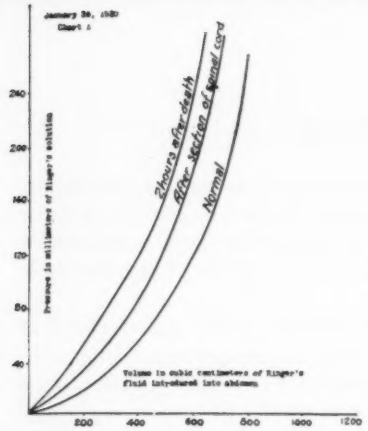
Chart 1
Pressure in millimeters of Ringer's solution
2 hours after death
After section of spinal cord
Normal
Volume in cubic centimeters of Ringer's fluid introduced into abdomen

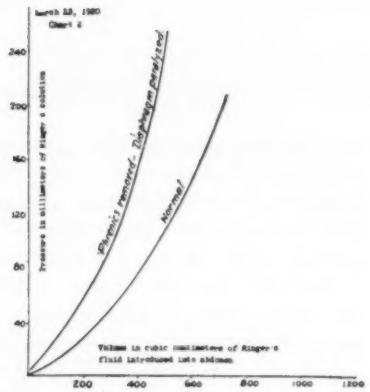
Chart 2
Pressure in millimeters of Ringer's solution
Phrenic rendered diaphragm paralyzed
Normal
Volume in cubic centimeters of Ringer's fluid introduced into abdomen

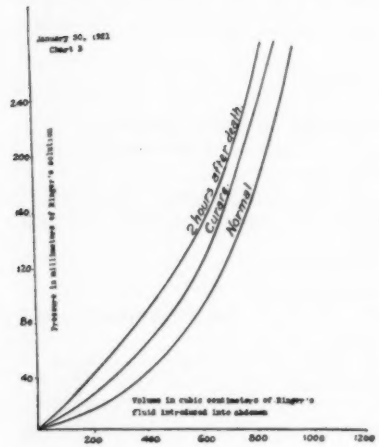
Chart 3
Pressure in millimeters of Ringer's solution
2 hours after death
Curare
Normal
Volume in cubic centimeters of Ringer's fluid introduced into abdomen

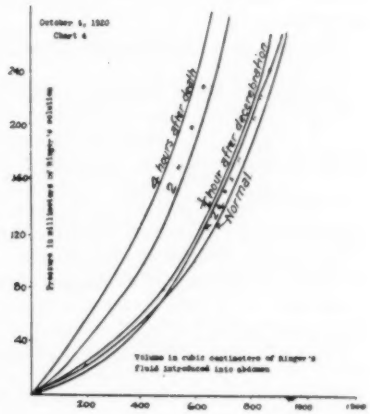
Chart 4
Pressure in millimeters of Ringer's solution
4 hours after death
2 hours after decerebration
Normal
Volume in cubic centimeters of Ringer's fluid introduced into abdomen

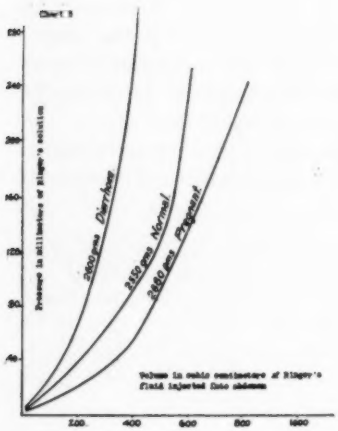
Chart 5
Pressure in millimeters of Ringer's solution
2650 gms. Diarrhoea
2350 gms. Normal
2080 gms. Pregnant
Volume in cubic centimeters of Ringer's fluid injected into abdomen

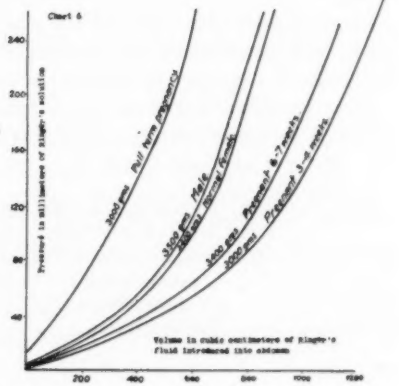
Chart 6
Pressure in millimeters of Ringer's solution
3400 gms. Full term pregnancy
3100 gms. Male
3000 gms. Normal female
2600 gms. Pregnant 6-7 months
2000 gms. Pregnant 3-4 months
Volume in cubic centimeters of Ringer's fluid introduced into abdomen

the obliqui, internus and externus and the transversalis abdominis, which are the antagonists of the diaphragm appears to lead to a greater increase of intra-abdominal pressure in proportion to the volume of fluid injected; that is, to diminish the reflex tonicity of the muscular portion of the abdominal walls (chart 2).

Probably the rôle played by the diaphragm in the maintenance of the tonus of the abdominal cavity is of great importance. In a comparative study of the physiological properties of certain striated muscles in the cat, Lee (16) has shown that of the five selected skeletal muscles under discussion, the diaphragm takes the longest to fatigue and possesses much more efficient muscular tissue than do the other muscles.

The curare experiments are next in order.

January 30, 1921. Male cat, weight 3000 grams. Ether, tracheotomy. Cannula in left femoral vein. Cannula in abdominal cavity, connected with recording apparatus.

10:00–11:20 a.m. 800 cc. Ringer's solution injected. Pressure 250 mm. of Ringer's solution. Respiration labored. Curve of intra-abdominal pressure taken.

11:25 a.m. Fluid removed from abdominal cavity.

11:40 a.m. Artificial respiration. Curare injected.

11:50 a.m.–1:10 p.m. 650 cc. Ringer's solution injected. Pressure 240 mm.

1:15 p.m. Fluid removed from abdominal cavity.

3:00– 4:00 p.m. Curve of relation of intra-abdominal pressure to volume of fluid injected taken after death.

Here we had abolition of all efferent nervous impulses affecting skeletal muscles but the alteration in the curve is no greater than when the afferent nervous mechanism alone is eliminated. This may be due in part to the mechanical conditions which prevail in the thorax with artificial respiration. The lungs being inflated to a regular degree with each respiration, the descent of the diaphragm is mechanically determined, even if the phrenics are sectioned (chart 3).

The question of the effect of decerebration upon intra-abdominal pressure was tried out in another series of experiments. Following is the protocol and curve for the experiment.

October 4, 1920. Female cat, weight 2600 grams. Ether, tracheotomy. Cannula in abdomen, connected with recording apparatus.

10:00–11:00 a.m. Ringer's solution injected, 700 cc. Pressure 240 mm. Respiration labored. Fluid removed.

11:20 a.m. Carotids tied off.

11:25 a.m. Decerebration.

12:00 m.–1:10 p.m.	700 cc. fluid enters abdomen, pressure 260 mm. of Ringer's solution.
1:15 p.m.	Fluid removed.
3:40– 4:50 p.m.	700 cc. fluid enters abdomen; pressure 250 mm. of Ringer's solution.
5:00 p.m.	Fluid removed.

From this experiment, it will be seen that after decerebration the curves obtained after intervals of half an hour and two and one-half hours respectively are so similar to the normal that we cannot ascribe any particular significance to decerebration in this connection (chart 4).

Various casual occurrences are of interest in the general picture. In one experiment micturition occurred about the middle of the control portion of the experiment. Intra-abdominal pressure fell from 160 mm. to 110 mm. of Ringer's solution. It is not a usual occurrence for even the highest intra-abdominal pressures to excite activity of the bladder, under ether.

With increased intra-abdominal pressure, there is a change in *rate* as well as in the character of respiration. In many cases, the number of respirations per minute diminishes proportionately with the increase in pressure. While costal respiration is greatly augmented in depth, the increase probably does not entirely compensate for the slowing in rate.

Observations were made in a number of experiments on the point at which the marked change from abdominal to costal respiration takes place. In most cases it was found to occur when the intra-abdominal pressure had risen to from 150 mm. to 180 mm. of Ringer's solution, or when about 500 cc. of fluid had entered the abdomen.

The question has frequently been asked whether systemic arterial blood pressure is affected by distention of the abdominal cavity with fluid. In the forty or more experiments in which arterial blood pressure was recorded, no appreciable rise in carotid blood pressure was ever obtained on the introduction of fluid. But in several cases, when the fluid was removed from the abdominal cavity, there was a distinct rise in arterial pressure (fig. 1).

It wa suggested that although, while the blood supply was normal, arterial pressure might be unaffected, under conditions of hemorrhage a change might take place. Several cats accordingly were bled, from 25 per cent to 40 per cent of the total volume of blood being drawn and the abdominal cavity was again distended with Ringer's solution. The results of two typical experiments may be tabulated as follows:

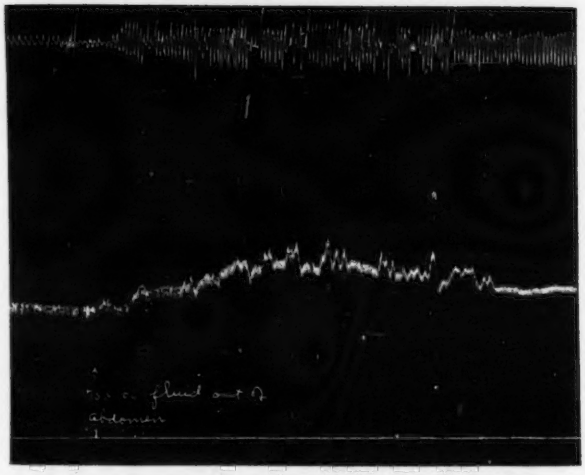

Fig. 1. The tracing shows the effect on costal respiration and carotid blood pressure when 700 cc. of Ringer's solution are released from the abdominal cavity. Note the change in respiratory rate and depth and the rise in blood pressure.

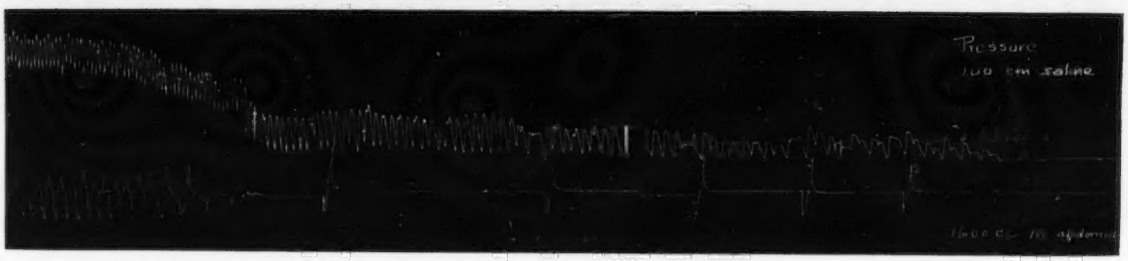

Fig. 2. The tracing shows the onset of respiratory failure before heart failure under conditions of excessive intra-abdominal pressure. Sixteen hundred cubic centimeters of Ringer's solution are in the abdominal cavity, and the intra-abdominal pressure is 1000 mm. fluid. Upper tracing, blood pressure; lower tracing, respiration.

DATE	CONTROL PRESSURE	BLOOD LOST	PRESSURE ON HEMORRHAGE	AFTER INJECTION OF FLUID	AMOUNT FLUID
	mm. Hg.	*cc.*	*mm.* Hg.	*mm.* Hg.	*cc*
July 10, '21	72	70	36	75	700
July 12, '21	75	40	58	75	450

In connection with this series of experiments I was able to demonstrate that under acute conditions of very high intra-abdominal pressure, respiration becomes dyspneic and almost fails before the failure of the heart. This is contrary to Emerson's statement that "Excessive pressure artificially produced within the peritoneal cavity causes death from cardiac failure before the obstruction to the respiratory excursion has developed a marked asphyxia." In cats, at least, the respiratory (fig. 2), disturbance is more marked before the onset of the cardiac failure.

Two interesting deviations from the normal were studied in the cats brought to the laboratory. During the cold weather of 1920, several of the cats used for experiments had winter cholera. In every case the intra-abdominal pressure was much higher in proportion to volume of fluid injected than in normal cats of the same size. While this may have been due to intestinal distention, one is reminded of the rigidity of the abdominal walls in cases of peritonitis. Conditions of disease have yet to be studied in their relation to certain nervous regulatory reactions. In any case, there was a remarkable inability of the abdomen to accommodate large volumes of fluid at a low pressure (chart 5).

In pregnancy, on the other hand, there was a much greater ability to accommodate fluid at a low pressure than in the non-pregnant cat. Following is a chart indicating the pressure curves at different stages of pregnancy. Obviously, at full term, the mechanical conditions would be such as to preclude very much regulation by the nervous mechanism, but from the second or third week through the sixth or seventh, the pressure is very much lower than would be proportionate to the volume of fluid injected in non-pregnant cats of the same weights (chart 6).

The lack of change in the pressure curve after decerebration might appear to put in question the possession by the abdominal muscles of that reflex tonicity known as "postural activity" which, in the case of the anti-gravity muscles, decerebration brings out so sharply as "decerebrate rigidity." Sherrington has expressed a doubt as to

abdominal pressure is 1000 mm. fluid. Upper tracing, blood pressure; lower tracing, respiration.

whether or not this particular form of reflex tonicity is present in the ventral muscles of the abdomen. It seems to us that these muscles demonstrate postural activity but not necessarily decerebrate rigidity, since they have been shown to exhibit that "reflex lengthening without change in tension" which is the fundamental definition of postural activity, and, on the other hand, to exhibit no departure from normal conduct upon decerebration. It would therefore appear that the phenomenon of postural activity is not strictly synonymous with decerebrate rigidity, for while decerebrate rigidity is undoubtedly one form of postural activity, postural activity does not necessarily imply decerebrate rigidity at all. If, as Starling stated in 1900, the respiratory center in the medulla governs to a large extent not only the activity of the diaphragm, but of the other abdominal muscles as well, why should we expect to find decerebrate rigidity in the abdominal muscles any more than in the muscles of respiration after decerebration? Yet there is little doubt that one can demonstrate postural activity as such of the muscles of respiration.

Some confusion may lie in the difficulty of determining to what degree the activity of this group of muscles is due to its reflex properties or to the inherent qualities of the muscle itself. We have the muscle in situ with all its nervous connections intact, concerning the state of contraction of whose cells we know little or nothing from histological study. Then, severed from its nervous mechanism, living muscle always contains some fibers in a state of relaxation while others are in a state of contraction. Lingle (18) demonstrated histologically that in whatever phase excised muscle is fixed, the cell nuclei show that some cells are in a state of contraction while others are not; and Pratt (17) has shown by an extension of the "all or none" principle to striated muscle that each fiber responds according to this law, but that there is a variation in the number of fibers which causes a degree of difference of contraction of the muscle as a whole.

Finally we have the elasticity of dead muscle in which the state of contraction or relaxation of its individual fibers does not enter into consideration.

In some cases a variation in the number of contracted muscle fibers would explain differences in the degree of contraction exclusive of any reflex mechanism. During pregnancy, hyperplasia of the uterus has been demonstrated, but hyperplasia of the abdominal musculature has not been shown. Since the abdominal walls must enlarge in some manner to accommodate the increased volume, and since intra-abdomi-

nal pressure is somewhat lower during pregnancy than under normal conditions, it follows that relaxation of the abdominal musculature must take place which would be readily accounted for in part at least by the evidence adduced by Pratt and Lingle.

With an agreeable unanimity, the pregnant cats throughout the entire series showed, in proportion to their size, an ability to accommodate a greater volume of fluid at a lower pressure than did the normal cats. The fact that they were very much more resistant to high intra-abdominal pressures than the normal cats, that it took a pressure of 800 mm. to 1000 mm. of Ringer's solution to produce labored respiration and heart beat instead of the much lower pressures observed in the non-pregnant cats, would appear to indicate the compensatory activity of the central mechanism. Just what percentage of the entire regulation is reflex, and what percentage is purely an intrinsic function of the abdominal musculature is more difficult to determine than in the case of the so-called anti-gravity muscles or the smooth muscle viscera, but while we must ascribe considerable activity to the musculature itself, we must acknowledge the intervention of a reflex nervous regulatory mechanism.

SUMMARY AND CONCLUSIONS

1. In the normal cat intra-abdominal pressure does not increase greatly in proportion to volume of fluid injected until a critical pressure is reached. At this critical pressure the character of the respiration becomes markedly costal.

2. The lack of increase in intra-abdominal pressure, up to a certain limit, in proportion to volume of fluid introduced may be considered to demonstrate that reflex tonicity of the abdominal musculature which is known as "postural activity."

3. The reflex nature of this mechanism is shown by the alteration of the pressure curve when any portion of the nervous mechanism involved is subjected to injury.

I wish to express my thanks to Prof. F. H. Pike of this department for his valuable suggestions and criticisms of this work.

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THE HEIGHT OF THE CENTER OF GRAVITY IN MAN

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The first attempts to determine the position of the center of gravity in man were made by Borelli (1). This investigator placed a naked subject in the supine position upon a plank which he shoved back and forth over a triangular prismatic bar until equilibrium was attained. When the plank and subject were nicely balanced, a vertical plane drawn upward through the supporting edge of the prism was held to contain the center of gravity of the body. From such experiments Borelli concluded that the center of gravity of the human body lay slightly below the pubis.

The method of Borelli was obviously crude and his results inaccurate but his work was very suggestive and was taken up and carried forward by Wilhelm and Edouard Weber (2). These investigators made several changes in Borelli's procedure. In the first place they balanced the plank separately and having noted its position when in equilibrium kept it constantly in that position. Subsequent adjustments were made by shoving the subject back and forth upon the plank. Furthermore they did not seek to balance the plank but noted the points at which it tipped now to one side, now to the other, and took the mean of these observations. Apparently they examined but a single subject. In him they found the center of gravity to lie in a plane 56.7 per cent of the distance from the soles of the feet to the crown of the head.

[1] The history of this research is as follows: In 1917 Luessen and Marohn determined the position of the center of gravity in two hundred subjects and embodied their observations in their thesis for 1918. In 1920 Croskey and Wright made an examination of Borelli's method on twenty subjects and embodied their observations in their thesis for 1921. In 1919 Dawson made the analyses of values supplied by Luessen and Marohn and in 1921 with Croskey wrote up the article as now presented. A summary of a part of this work has appeared in the transactions of the American Physiological Society, This Journal, 1921, lv, 312.

The next investigation in which the method of Borelli was employed was that of Hermann Meyer (3). Meyer found that in a subject whose length was 189.7 cm. the center of gravity was 59.514 per cent of the distance from the soles to the crown. This placed the center 2.73 per cent of the total body height higher (cephalad) than the Weber brothers.

In 1857 Harless (4) made a series of observations upon four subjects. The mean distance of the center of gravity from the soles was 57.993 per cent of the total body length, a value differing from that given by the Weber brothers by only 1.293 per cent.

In the investigation of Braune and Fischer (5) a new method was devised. These observers froze a cadaver in the supine position. When the body had become perfectly rigid, it was transfixed by three spits each at approximately right angles to the other two. The body was then suspended by each spit in turn. The three vertical planes each of which included one of the three spits all contained the center of gravity of the cadaver and consequently their point of intersection coincided with the center of gravity itself. Subsequently the bodies were sawn apart and the exact loation of the center determined. In only one of the three cases studied by Braune and Fischer in this way are data furnished from which it is possible to estimate the distance of the center of gravity from the extremities of the body. In this case it was 54.8 per cent of the distance from the soles to the crown of the head.

Up to the present there has been no extensive series of observations and no attempt made to discover differences corresponding to various types of physical development as, for example, that existing between men and women. To extend our information on this subject the observations were made which form the subject of this paper.

METHOD: *Description of method.* It is to be remembered that in Borelli's experiments, both plank and subject were shoved back and forth over the fulcrum, and that the Webers improved upon this technique by balancing the plank first and keeping it in position while shoving the subject back and forth. We have added further to the convenience of the apparatus by having the fulcrum screwed to the plank (instead of being fastened to the supporting table) so that the plank could be balanced once for all. Moreover the upper surface of the plank was marked off with parallel cross lines 5 cm. apart and the making of measurements was further facilitated by a simple wooden frame which carried a vertical surface and in structure and use resembled the sliding part of the shoemaker's measure with which he measures the length of one's

feet, or the sliding part of the scale used commonly in gymnasia to measure one's height when standing or sitting.

When the tipping point had been determined, this indicator was slid along toward the subject until the flat surface rested against one or the other of the extremities of the subject's body and the distance from the nearest cross line on the plank was then measured with a small scale held in the observer's hand.

Although in every case the subjects tested upon this see-saw were supine, the position occupied by their extremities was varied in four different ways. In the first position, called in this paper *n* (natural), the traditional attitude was assumed, the legs being extended and the arms, also extended (toward the feet), being pressed close to the sides of the body. In the second position, *k* (knees), the knees (and thighs) were drawn up (flexed) and the soles pressed downward against the plank, but otherwise the subject remained as in *n*. In the third position, *a* (arms), the arms were extended as far above the head as possible. Perhaps, as the subject was supine, one should say "beyond" rather than "above." In other respects the subject remained as in *n*. The fourth position was a combination of the second and third and consequently designated *ka*. Here the knees were drawn up and the arms extended above (i.e., beyond) the head.

In this article the position of the center of gravity is always stated in terms of the percentage of body length at which it lies above the soles when the subject lies in the position designated natural, *n*. If the arms be extended above the head, the center of gravity moves in the body cephalad and its distance from the feet is increased. When the feet are drawn up through flexion of the thighs and knees, *k*, the center of gravity is again moved toward the head but on account of the drawing up of the legs its distance from the feet is not increased. If then we are to observe a constant procedure and to have our measurements comparable, we must *assume* that the legs are always extended and measure either from the soles or from the position which the soles would occupy if the legs were extended as in the so-called natural position. If then we say that on extending the arms or flexing the knees the center of gravity has risen say 3 per cent, what is meant is 3 per cent of the length of the body in the natural position, while by the statement that its position has risen from say 53 to 56 per cent is meant 53 and 56 per cent of the distance from the soles to the crown if the subject were in the natural position. The calculations necessitated by the above procedure were facilitated by use of some simple and obvious formulae.

Analysis of method. Now although for 300 years Borelli's method has been accepted as adequate for determining the position of the plane passing through the center of gravity at right angles to the axis of the body, yet there have been no systematic studies of the accuracy of this method, of the sources of error and of the importance of the latter. It was to supply this need that two of us (6) undertook the following analysis of the sources of error which one is most likely to encounter in employing Borelli's method with the subject consecutively in all four positions.

After some preliminary observations on two individuals it was decided to investigate the following possible sources of error: *1*, the effect of a full meal; *2*, the effect of clothing; *3*, the effect of carelessness of the subject in assuming the position requested. For, in connection with the source of error last mentioned, it had been found that the feet were sometimes flexed and sometimes slightly extended in positions *n* and *a*; that the degree of flexion of the legs varied in positions *k* and *ka*; and finally that the degree of extension of the arms varied in positions *a* and *ka*.

It was of course necessary to select a standard from which to measure departures due to the sources of error above mentioned. The standard adopted involved the following precautions; *a*, the shoes were removed (but all other clothing was retained); *b*, in positions *n* and *a* the ankles were so flexed that the plane of the soles of the feet was at right angles to that of the plank, in *k* and *ka* the knees were flexed as completely as possible with the assistance of the operator; in *a* and *ka* the arms were stretched as far as possible above the head, elbows, wrists and fingers being in complete and therefore uniform extension.

The effect of a full meal. Observations were made in all four positions (*n*, *k*, *a* and *ka*) immediately before and within an hour after the midday meal. All the requirements of the standard conditions were carefully attended to, so that the only variable was the weight of the stomach and the redistribution of the blood (see Mosso, 7). Five observations made on each of three subjects showed no appreciable alteration in the position of the center of gravity due to this cause. On this account the observations were discontinued.

Effect of clothing. The subjects, twenty in number, upon whom observations were continued, were women students chiefly from the school of physical education. As already stated they were examined in all four positions with and without clothing. The effect of the wearing of clothes was usually negative (lowering the center of gravity)

showing that it was chiefly due to skirts. This, however, would depend very much upon the type of clothing worn. In the present series most of the subjects wore heavy plaited skirts and light waists. The amount of the error due to clothing was found to be for the most part very small indeed (0.23 per cent).

In position *n* there were 4 cases in which there were no errors at all; of the remaining 16 cases 10 were negative (average error 0.2) and 6 positive (average error 0.3); the greatest error was 0.5 and the average absolute deviation 0.21. (In this connection it seems appropriate to inform the reader, who may be unfamiliar with such terms as "average deviation," "standard deviation," and so forth, that he will find a lucid and sufficiently full explanation of both the terms and the processes of statistical analysis in *A Biometric Study of Basal Metabolism:* Harris and Benedict, chapter II., Carnegie Institution, 1919.)

In position *k* 15 cases were negative (0.3) and 5 positive (0.2); the least error was 0.1, the greatest 0.8 and the average absolute deviation 0.2.

In position *a* there were 6 cases in which there were no errors at all; of the remaining 14 cases 10 were negative (0.43) and 4 positive (0.3); the greatest error was 0.9 and the average absolute deviation 0.2.

In position *ka* there were 2 cases in which there were no errors at all; of the remaining 18 cases 17 were negative (0.4) and 1 positive (0.1); the greatest error was 1.2 and the average absolute deviation 0.3.

Note that all the values given above are percentages of the "standard" body length that is, of the body length in position *n*.

Effect of careless posture. The same twenty subjects were then examined in all four positions under the following circumstances: *1*, with care as to the amount of flexion of the knees in positions *k* and *ak*; *2*, with care as to the amount of extension of the arms in positions *a* and *ak*; *3*, with care as to position of the feet in positions *a* and *n*. The results of this study are shown in table 1.

When the writers perused the data of which table 1 is a summary, they were unable to discover any connection between the physical type of individual and the amount or chance of error, except that those showing the least error were generally those who show skill in athletics and gymnastics, while those showing the greatest error were among the less skilled. The individual whose physical coördination is high takes the desired positions accurately without admonitions, while other individuals do not.

Examination of the table itself leads to the following conclusions.

For *Fn* and *Fa*, the occurrence of negative as compared with positive error is as 12 to 5, and the average negative error (0.385) is greater than the average positive error (0.17). An inaccurate position of the feet then is most likely to lower the center of gravity as would be expected.

The five cases showing a rise may be accounted for by the fact that in allowing the foot to take an easy and natural position, the knees are slightly flexed, thus more than compensating the effect of extension of the ankle. The chance of error is exactly equal in these two positions (17:3 or 5.6:1).

TABLE 1

Shows errors due to conditions enumerated under "position." Sources of error are designated by capital letters and positions by small letters. Thus F indicates that all conditions are standard except feet, which may or may not have been flexed to a right angle at ankle joint. Errors occuring under F are therefore due to feet only. Mutatis mutandis the same applies to K and A. Thus in K source of error is varying completeness of flexion of lower extremities; in A, of extension of upper extremities. S (standard) indicates that all sources of error have been eliminated. When in natural position (n) placing of feet is disregarded (F), results differ from those obtained when feet are carefully placed (S) in direction and to degree described in figures 0.32 per cent, −0.8 per cent, etc. For discussion, see text.

POSITION	AVERAGE DEVIATION	GREATEST ERROR	LEAST ERROR	NEGATIVE		POSITIVE		NO ERROR
				Number cases	Average	Number cases	Average	Number cases
Fn-Sn	0.32	−0.8	0.0	12	0.45	5	0.20	3
Fa-Sa	0.23	−0.8	0.0	12	0.32	5	0.14	3
Kk-Sk	0.44	−0.9	−0.1	20	0.44	None		0
Kka-Ska	0.42	−0.1	−0.1	18	0.43	2	0.25	0
Aa-Sa	0.30	−0.8	0.0	7	0.20	12	0.20	1
Aka-Ska	0.20	−0.6	0.0	11	0.28	6	0.20	3

The chance (100 per cent) and average amount (0.43) of error due to incomplete flexion of knees is greater than those due to any other condition (e.g., 5.6:1 and 0.275 in *F*). There are no positive variations in *Kk* and but two in *Kka* in which the average negative error (0.43) is almost twice as large as the average positive error (0.25).

The average error due to incomplete extension of the arms overhead (0.25) is less than that due to condition *F* (0.275); still less than that due to *K* (0.43). When *Aa* and *Aka* are combined the error is about equally divided between positive and negative variations (9:9). The occurrence of negative to positive variation in *Aa* is as 12 to 7 (or 7.1:1) and in *Aka* 6:11 (or 0.55:1). This unexpected difference may be explained as follows. In extending the arms either completely or incompletely above the head the chest may remain stationary or be pulled

up. In the latter case the center of gravity will be raised. This factor was not controlled.

In *F*, *K* and *A*, the greatest average variation occurs in the position involving the natural position of one pair of extremities (cf. 0.32 0.44, 0.30 and 0.23, 0.42, 0.20). That is to say, error due to extension of the feet is greater when the arms are at the sides than when they are extended overhead. It may be that when the arms take a definite and accurate position the feet and legs also tend to take a more accurate position than would otherwise be the case and *vice versa*, as though the whole body were called to attention by the effort to adjust a part. The chance of error is 17 in 20 for errors due to *F*, 18 in 20 for *A*, and 20 in 20 for *K*.

These conclusions may be summarized[2] as follows:

1. The average error (deviation) due to *clothing* is 0.23 per cent of the body length.
2. It is usually negative, i.e., places the center of gravity unduly low (caudad).
3. The average error (deviation) due to *inaccuracy* in assuming a required position is 0.32 per cent of the body length.
4. The error due to this inaccuracy is greater with the physically untrained than with trained subjects.
5. It is greater in positions involving flexion of the knees.
6. It is usually negative, i.e., places the center of gravity unduly low (caudad).
7. It is greater when one pair of extremities is in the natural position.
8. It is greater than the error due to clothing.
9. The *chance of error* due to clothing is equal to that due to inaccurate position of the feet (17:3 or 5.6:1 in each case).
10. It is greatest (100 per cent) in positions involving flexion of the knees, and least in those involving position of the feet (17:3 or 5.6:1).

RESULTS: Series I (observations of Croskey and Wright). The special interest attaching to this series lies in the fact that from it all the sources of error discussed under "Method" were carefully eliminated. It is, however, open to the objection that it comprises only twenty subjects. In view of the latter fact it has not been subjected to the same statistical analysis employed in the case of series II but is presented more briefly.

[2] It should not be forgotten that these conclusions are drawn from a study of women only.

The physical characteristics of this series in regard to the average weight and height were 161.5 cm. and 60.9 kilos respectively. The location of the center of gravity in position *n* was 56.5. The displacement (rise) in percentage of standard body length was in position *a* 3.25; in *k*, 3.75; and in *ka*, 6.75.

From these values it is seen that the sum of the average rise due to flexion of the knees, *k*, and that due to extension of the arms *a*, is 0.25 per cent (of the standard body length) higher than the average rise due to the combination of these two positions, *ka*. This may be explained by the fact that when the arms are extended overhead without flexion of the knees, *a*, the back is hollowed and the capacity of the mid-abdominal region decreased so that the organs slide upward toward the diaphragm and thus the center of gravity is raised. But in the position *ka* flexion of the knees keeps the back flat. The smallness of this difference is of importance for the following reason. In series II the subjects were not examined in the *ka* position but the writers have added the rise of the center of gravity obtained in the *a* position to that obtained in the *k* position and present these values instead. Such a procedure requires some justification and the latter is found, to some extent at least, in the fact stated above, namely, that the difference in question is a small one.

An attempt was made (see table 2) to correlate at least qualitatively the physique of the subject and the degree to which the center of gravity is shifted by change of position. The greatest rise due to assuming position *k* is in Albrecht, who is the tallest in the group and has long legs and large feet, while the least rise is shown in Henry and Shepard both of whom have short legs and thick trunks. The same may be said of Bruno and Bradley who show the least change due to position *a*, while the greatest change due to this position is found in Kropf and Tilton who are slender with long extremities. Position *ka* again shows the greatest change in Albrecht and Kropf and the least in Henry.

Series II (observations by Luessen and Marohn). The subjects of this series were one hundred in number, fifty men and fifty women. They comprised students, instructors and soldiers, the majority of whom were between 19 and 25 years of age. The weight and height of each subject were determined and the values thus acquired were submitted to a brief statistical analysis for the purpose of obtaining the average, the distribution, the standard deviation and the coefficient of variation, for both sexes taken together and for each sex taken

separately. The results, which are given below, show that in respect to weight and height the group is in no way atypical.

In respect to weight the averages for men and women taken together and for men and women taken separately are 62.11, 65.88 and 59.38 kilos respectively. The standard deviation, σ_w, was for these same three groups $\pm$ 6.773, $\pm$ 5.227 and $\pm$ 6.54 respectively, and the coefficient of variation, V_w, $\pm$ 10.909, $\pm$ 7.937 and $\pm$ 10.11 respectively. In order to determine the distribution, the entire series was divided into seven sub-groups. The first comprised those persons whose weight was less than 50 kilos; the second 50 to 55 kilos; and so forth. When the numbers of men and of women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 0 men, 2 women; second, 1, 11; and for the remaining sub-groups 5, 15; 18, 14; 23, 6; 3, 1; and 0, 1 respectively.

TABLE 2

Shows displacement cephalad of center of gravity in passing from normal position to other positions mentioned in table. Those who show greatest displacement have longest extremities as stated in text.

POSITION	CHANGE OF CENTER OF GRAVITY (RISE) IN PER CENT OF BODY LENGTH				
	Average	Maximum		Minimum	
	Per cent	Per cent	Subject	Per cent	Subject
k	3.75	4.9	Albrecht	2.9	Shephard Henry
a	3.25	3.8	Kropf Tilton	2.8	Bruno Bradley
ka	6.75	7.8	Albrecht Kropf	5.2	Henry

In respect to height the averages for men and women taken together and for men and women taken separately are 168.40, 173.32 and 163.49 cm. respectively. The standard deviation, σ_h, is for these same three groups $\pm$7.9, $\pm$5.55 and $\pm$7.594 respectively, and the coefficient of variation, V_h, $\pm$4.68, $\pm$ 3.20 and $\pm$4.64 respectively. In order to determine the distribution the entire series was divided into ten sub-groups. The first comprised those persons whose height fell below 145 cm.; the second those whose height fell between 145 and 150; and so forth. When the numbers of men and women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 0 men, 1 woman; second, 0, 0, and for the remaining sub-groups, 0, 2; 0, 14; 3, 12; 12, 10; 16, 9; 12, 2; 6, 0; and 1, 0 respectively.

The apprehensions which led Croskey and Wright (8) to study the sources of error in Borelli's method were not felt when this series of

cases was being examined. Consequently only a few precautions were taken to insure accuracy. These were in respect to the condition of flexion of the ankle joint, which was carefully controlled, and in respect to the shoes. The latter were removed in every case, our special objection to this part of the clothing being first, that since they were placed near the end of the balancing board their leverage was great, and secondly, that they were often very heavy especially in the case of the men. No other precautions were adopted except of course such as were perfectly obvious but the studies of Croskey and Wright (already considered in this paper) were such as to convince us that there was really very little danger of error to be feared and that the observations of series II would not have been altered by more than a small fraction of a per cent of the body length by taking every precaution as yet thought of. It should be emphasized, however, that no very high degree of accuracy was either expected or sought. Previous investigators have sometimes expressed their results in hundredths of a millimeter but such a procedure is no indication of accuracy and was probably not so intended by the investigators in question.

The determinations of the center of gravity of the subjects of this series were subjected to a partial statistical analysis for the purpose of obtaining the average, the curve of distribution, the standard deviation and the coefficient of variation. These results are shown in table 3 and figure 1.

The next step was to determine the coefficients of correlation between the weight and height and the position of the center of gravity. The values obtained are also given in table 3, which shows that there is no significant correlation present between the position of the center of gravity and the other values mentioned.

The results so far presented deal only with the center of gravity of the subjects when lying in the position which we have designated natural, *n*. With the subjects in position *a* the average increase of the body length was 21.76 per cent of the "standard" body length (22.06 in men and 21.44 in women). This increase is usually between 19 and 25 per cent in men and also in women; but the increase in the latter is more variable in amount.

In order to determine the distribution the entire series was divided into ten sub-groups. The first comprised those persons whose increase in length on assuming position *a* was 9 per cent; the second, 11 per cent; and so forth. When the numbers of men and women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 0 men, 1 woman; second, 0, 3; and for the remaining sub-groups, 0, 1; 1, 0; 1, 2; 12, 5; 7, 8; 14, 18; 15, 9; and 0, 3 respectively.

TABLE 3

Shows in respect to the center of gravity of subjects the distribution, average, standard deviation, σ_c, coefficient of variation, V_c, and coefficients of correlation with weight and height. Note small difference between the averages in men and women and also greater variability in the case of the latter. Note also absence of acceptable degree of correlation.

CENTER OF GRAVITY PER CENT	NUMBER OF SUBJECTS		
	Both	Men	Women
Distribution:			
54	9.0	0.0	9.0
55	27.0	9.0	18.0
56	41.0	24.0	17.0
57	20.0	16.0	4.0
58	3	1.0	2.0
Average position	55.81	56.18	55.44
σ_c	±0.9554	±0.234	±1.09
V_c	±1.710	±0.416	±1.969
Coefficients of correlation:			
Weight	0.01	0.54	−0.12
Height	0.14	−0.45	−0.05

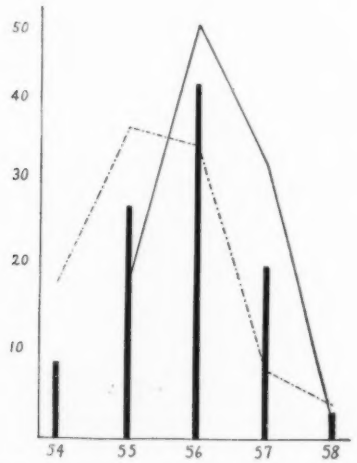

Fig. 1. Showing distribution of center of gravity in percentages of distance from sole to crown. Solid line represents men; broken line, women; and heavy vertical lines, all subjects irrespective of sex. Distances along the abscissa indicate distance, in percentage of ("standard") body length, of center of gravity from so'es of feet. Distances in vertical column indicate number of subjects in percentages. *Note the greater variability in women.*

The effect upon the center of gravity of raising the arms above the head (position *a*) is to raise this center (average 3.2 per cent). This rise is somewhat higher in men (3.5) than in women (2.9), and among the latter the individual variations are greater. The distribution of these changes is shown in figure 2.

In order to determine the distribution, the entire series was divided into five sub-groups. The first of these comprised those persons whose center of gravity on assuming position *a* was raised 1 per cent of the "standard" body length; the second, 2 per cent; and so forth. When the numbers of men and women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 0 men, 4 women; 0, 10; and for the remaining sub-groups, 26, 21; 23, 15; and 1, 0 respectively. These are the values used in constructing figure 2.

With the subjects in position *k* the average decrease of the body length was 21.09 per cent of the "standard" body length (21.18 for men and 21.00 for women). This decrease is usually between 18 and 24 in men and also in women, but the decrease in the latter is more variable in amount.

In order to determine the distribution, the entire series was divided into ten sub-groups. The first of these comprised those persons whose decrease in length on assuming position *k* was 10 per cent; the second, 12 per cent; and so forth. When the numbers of men and women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 0 men, 3 women; second, 0, 0; and for the remaining sub-groups, 0, 1; 1, 3; 12, 5; 22, 13; 8, 13; 4, 10; 1, 2; and 2, 0 respectively.

The effect upon the center of gravity of flexing the legs (position *k*) is to raise the center an amount (4.5) which is greater than that due to extension of the arms, *a*. This rise is somewhat higher in men (4.9) than in women (4.1). As a rule the rise in both sexes is between 3 and 5 per cent. The distribution of these changes is shown in figure 3.

In order to determine the distribution the entire series was divided into five sub-groups. The first of these comprised those persons whose center of gravity on assuming position *k* was raised 2 per cent of the "standard" body length; the second, 3 per cent; and so forth. When the numbers of men and women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 1 man, 3 women; second, 10, 12; and for the remaining sub-groups 29, 15; 8, 17; and 6, 3 respectively. These are the values used in constructing figure 3.

In series II, as already stated, no observations were made with the subject in the position *ka* but, in lieu of any direct observations, the

rises produced by assuming positions *a* and *k* were addded together. Such a procedure is at least partly justified by the observations of Croskey and Wright as already stated. The values obtained in this way are 7.30 for the average rise of the center of gravity in percentage of the "standard" body length (7.50 in men and 7.10 in women). Here again the variability is much greater in women than in men, as shown in figure 4. For the most part the values fall between 6 and 8 per cent.

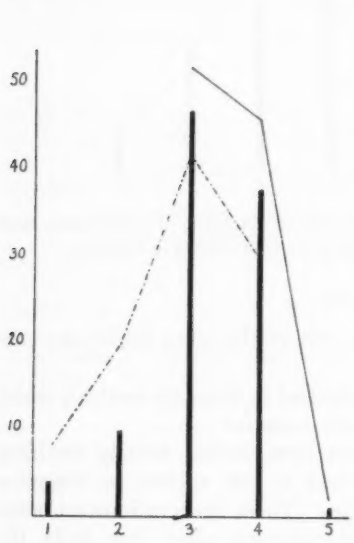

Fig. 2

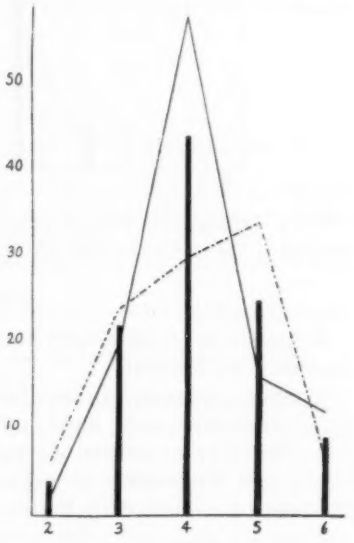

Fig. 3

Fig. 2. Showing effect of raising arms above head in raising the position of the center of gravity For further explanation see legend of figure 1. *Note that the extension of the arms produces less change than flexion of the legs* (cf. fig. 3) *and that the raising of the center of gravity is greater in men than in women.*

Fig. 3. Showing effect of flexion of legs in raising position of center of gravity. For further explanation see legend of figure 1. *Note that usual rise is between 4 and 5 per cent in both sexes; also that amount of displacement with flexion of legs is greater than with extension of arms.*

In order to determine the distribution, the entire series was divided into eight sub-groups. The first of these comprised those persons whose center of gravity in position *a* gave when added to that in position *k* an amount equal to 3 per cent of the "standard" body length; the second, 4 per cent; and so forth.

When the numbers of men and women falling into each of these sub-groups were counted, they were found to be as follows: First sub-group, 0 men, 1 woman; second, 0, 1; and for the rest of the sub-groups 1, 4; 9, 14; 16, 8; 15, 12; 6, 8; and 3, 2 respectively.

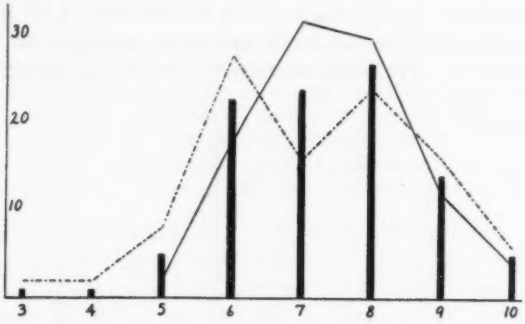

Fig. 4. Showing rise of center of gravity due to extension of arms above head plus rise due to drawing up legs. *Note much greater variability in women.*

SUMMARY

A statement of what seem to the writers the more important conclusions is as follows:

1. Some improvements have been devised in Borelli's method, which make observation more rapid and more accurate.

2. The following sources of error have been studied, namely, clothing, meals, and carelessness or awkwardness of the subject in assuming the position requested by the operator. These observations are summarized on page 177; the errors in question affect but little the results of this research.

3. Observations made upon fifty men and fifty women show a difference in the location of the center of gravity of less than 1 per cent of the body length. It is higher in men and more variable in women.

4. There was no acceptable correlation found between the weight or height and the center of gravity.

5. Extending the arms above the head increases the body length equally in men and women but the figures in the latter are more variable. This procedure raises the center of gravity more in men than in women and the figures for the latter are again more variable.

6. Flexion of the legs decreases the body length equally in the two sexes but the rise in the center of gravity is greater in men.

7. Flexion of the legs is more effective in raising the center of gravity than is extension of the arms.

8. Extension of arms and flexion of legs raises the center of gravity slightly higher in men than in women and the results in the latter are more variable.

9. The amount of change in the position of the center of gravity on extending the arms or flexing the legs or both depends upon the physique. In those with long and large extremities the effect of changing the position of the latter is naturally greater than in persons characterized by long and thick trunks.

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THE EFFECT OF PLETHORA AND VARIATIONS IN VENOUS PRESSURE ON DIASTOLIC SIZE AND OUTPUT OF THE HEART

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In dogs the heart has been found to retain its usual diastolic size and output until the blood lost by hemorrhage equals about 2.1 per cent of the body weight (1). This result has naturally suggested questions regarding the influence exerted by an increase in blood volume and subsequent hemorrhages. The present paper, therefore, deals with the effect of intravenous infusion on cardiac size; the influence of hemorrhage on a heart after such an infusion; and the relation of venous pressure to cardiac size and output.

Methods. The general plan for the experiments was similar to that reported in our preceding paper. Dogs were used in all experiments. The animals were morphinized and the experiment carried out under ether anesthesia.

The diastolic size of the heart was determined from x-ray photographs. The pictures were taken during successive inspirations with two or three short flashes from a Coolidge tube. The distance from tube to plate was kept at 1 meter which is sufficient to prevent serious distortion, corrections being made for that which did occur. In most cases it was sufficient for our purpose to know whether the heart size was increasing or decreasing relative to the normal, and this information was readily obtained by comparing the silhouette areas which had been measured by the planimeter. To determine the volume of the heart, which was necessary in computing ouputs, a formula expressing the relationship of the silhouette area of the dog's heart to its cubical contents, which has been developed by Skavlem (2), was used. According to his investigations the formula

$$\text{Volume of heart in cc.} = 0.44 \text{ Silhouette Area}^{3/2}$$

is sufficiently accurate for experimental work.

The solutions for producing plethora and raising venous pressure were injected normally into the left jugular, but at times into the femoral

TABLE 1

Data from experiments showing the effect on diastolic heart size of intravenous injections. Injections began at the time noted. The x-ray was taken either immediately after, in case venous pressure had not risen, or a little later when it had returned to normal

NUMBER OF EXPERIMENT	WEIGHT	TIME	INJECTION	PERIOD OF INJECTION	PULSE RATE	HEART AREA	TOTAL INJECTIONS IN CC. AND PER CENT OF BLOOD VOLUME
	kilos		*cc.*	*minutes*		*sq. cm.*	
H 79	8.5	4.03			140	34.4	320 cc. acacia
		4.22	50	12	126	37.6	
		4.36	50	6	144	36.8	38 per cent
		4.55	50	10	180	35.5	
		5.14	100	10	168	36.2	
		5.30	70	10	174	36.4	
H 80	6.0	2.50			48	46.8	250 cc. acacia
		3.00	50	6	60	47.7	
		3.56	50	6	48	46.9	43 per cent
		4.11	50	6	51	45.0	
		4.30	50	6	60	46.4	
		4.46	50	5	54	45.0	
H 81	8.5	3.07			78	48.5	350 cc. acacia
		3.26	50	6	48	50.0	
		3.41	100	10	70	52.8	42 per cent
		4.01	100	10	72	5.25	
		4.25	100	10	108	52.9	
H 82	7.0	2.45			54	53.0	300 cc. acacia
		2.50	50	6	42	52.9	
		3.28	50	6	42	54.1	44 per cent
		3.42	100	10	60	54.5	
		4.02	100	11	60	55.6	
H 83	6.8	3.15			72	38.5	500 cc. acacia
		3.24	100	10	60	38.2	
		3.42	100	10	66	38.9	76 per cent
		4.00	100	10	72	39.1	
		4.18	100	10	96	39.7	
		4.40	100	10	96	39.7	
H 84	12	2.26			66	62.7	1200 cc. of NaCl
		2.30	150	15	78	67.5	
		2.56	150	10	72	66.6	103 per cent
		3.23	150	10	84	67.0	
		3.40	150	12	96	69.0	
		3.56	200	10	90	67.6	
		4.16	200	12	108	63.4	
		4.35	200	13	126	71.4	

TABLE 1—*Concluded*

NUMBER OF EXPERIMENT	WEIGHT	TIME	INJECTION	PERIOD OF INJECTION	PULSE RATE	HEART AREA	TOTAL INJECTIONS IN CC AND PER CENT OF BLOOD VOLUME
	kilos		*cc.*	*minutes*		*sq. cm.*	
H 85	8.2	3.05			84	46.0	800 cc. of NaCl
		3.10	200	15	102	45.0	
		3.31	200	15	108	46.8	100 per cent
		3.56	200	15	114	47.2	
		4.20	200	15	90	43.6	
H 86	14.7	2.00			48	74.5	600 cc. of acacia
		2.35	300	25	54	76.2	40 per cent
		3.17	300	22	100	74.5	
H 87	8.0	3.30			63	46.0	320 cc. of acacia
		3.41	160	15	60	47.8	41 per cent
		4.19	160	23	96	45.7	
H 88	8.0	4.45			78	52.0	200 cc. of blood
		4.51	200	6	132	37.9	25 per cent
		5.50			120	44.6	
H 89	5.3	3.34			42	40.1	225 cc. of blood
		3.41	75	1	42	38.5	43 per cent
		4.31	150	2	51	39.5	

vein. Venous pressures were read from a sound inserted into the right jugular as far as the opening into the auricle. The sound was filled with 2 per cent citrate in physiological saline and balanced against a manometer containing the same solution.

Diastolic size of the heart after intravenous injections. Our first series of experiments was devoted to finding the effect on diastolic heart size of increasing the blood volume. A condition of artificial plethora was induced by injecting physiological saline, 6 per cent acacia in saline, or blood, into the femoral vein. The amounts injected varied from time to time as did the rates of infusion. X-rays were taken after each injection and at the end of the experiment. Enough time was allowed to elapse in each case so that transitory disturbances, such as a rise in venous or arterial pressure, had no particular effect. Pulse rates were carefully noted and in the majority of the experiments manometers recorded the arterial and venous pressures.

Table 1 gives the data from 10 such experiments. It will be noted that in no case did the heart area, as expressed in square centimeters,

show any increase proportionate to the amounts injected. Other experiments might be added to this table if it were necessary. The silhouette area of the x-ray shadow bears a definite relation to the volume of the heart. It is evident from the table then that the intravenous injections, although in two of the experiments the fluid injected equalled the original blood volume, did not materially increase the diastolic size of the heart. The results were uniform when either physiological saline, 6 per cent acacia in saline or blood was used as an injection fluid.

In one experiment only, H 84, was there at any time a really significant change in the silhouette area of the heart. The last injection it will be noted increased the area 8 sq. cm., a figure beyond the limits of error for the measurements. In H 81 there may possibly be a slight definite increase above normal.

In experiment H 88, in which blood was injected, there was a definite decrease in heart size. For some unknown reason the blood used for transfusion in this case seemed incompatible. Mean arterial blood pressure fell from 120 to 60 and with this change the heart no longer filled so completely.

That from 30 to 100 per cent of the blood volume may be added to the circulation without causing a dilatation of the heart is of course a remarkable fact. The first suggestion that comes to mind is that transudation has kept pace with the intake and that there is no real increase in volume. That this is not true, however, is evidenced by the absence of edema and any considerable amount of fluid in the body cavity except in those experiments where large amounts of pure saline were used. Acacia solutions up to 50 per cent of the blood volume gave little sign of increased transudation. Furthermore the hemoglobin readings showed that there was a real plethora. In experiments H 86, H 87, H 88, the hemoglobin determinations at the end of the experiment indicated blood volume increases of 28, 21 and 30 per cent respectively. Compared with the amount injected there was of course a loss, probably to the tissue fluids, but the net result was a marked increase in the total quantity of fluid in the blood vessels.

Since the fluid in the circulatory system had actually been increased without any cardiac dilatation, it was natural to seek for the excess in some other part. Arterial pressures showed no rise commensurate with the injections and so spoke against any increased content of the arteries. Neither was there any noticeable stasis in the venous cisterns. By elimination, and by our previous work showing that there

is normally excess fluid held in the capillaries and venules, these came to mind. Two experiments were therefore run in which the superficial blood vessels of the ear were studied before and during an injection. Hooker's (3) method of photographing the vessels was used. In both cases injection of fluid opened up empty channels and caused distention in those already present. Figure 1 shows photographs of the same field before and after an injection. The dog weighed 5.6 kilos and received 200 cc. of a 6 per cent acacia solution rapidly. Focus and illumination were unchanged. The exposures were of equal dura-

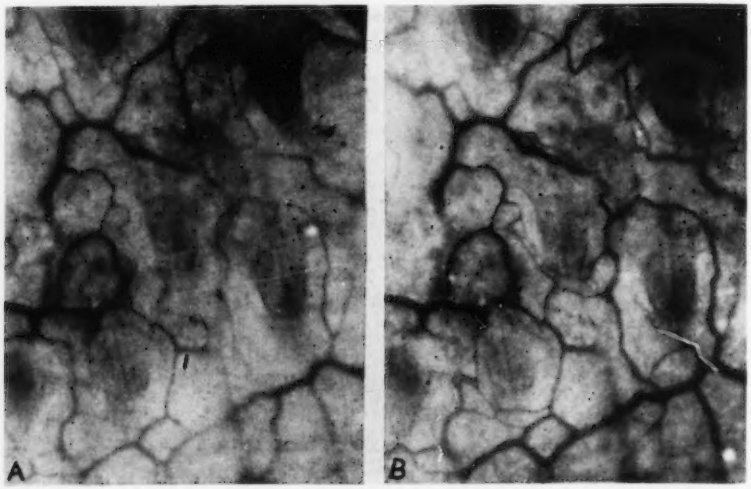

Fig. 1. Photomicrographs of the surface of a dog's ear before, *A*, and after, *B*, the injection of 200 cc. of acacia saline. Magnification 90×. Plates not retouched.

tion and the negatives were not retouched. It is evident that in figure 1B a number of new vessels have opened up and others have enlarged. We feel confident then that the heart is not dilated by the injection even of large quantities of fluid into the circulation because the excess is taken care of in the capillaries and venules.

So far as we know, this is the first demonstration of this mechanism, although it is interesting to know that its existence was postulated by workers in Ludwig's laboratory many years ago. Worm-Müller (4) found that intravenous injections would raise arterial blood pressure

only to a certain level in dogs and beyond this point further fluid had little or no effect. Since there was little evidence of distention in the venous systems and the autopsy showed little fluid in the body cavities, he suggested that probably the capillaries, especially those of the liver and lungs, were acting as reservoirs for the excess. Our experiment quite confirms this point of view.

If the capillaries and venules store injected fluid, it would seem possible in time to fill them completely and at this point further injections should distend the heart. It is probable that this very thing happened in experiment H 84 in which the last injection of 200 cc. very decidedly increased the diastolic size of the heart. It seems, however, that this cannot often be accomplished, for with increasing injections the transudation begins to accelerate and soon equals the inflow.

That injections within ordinary limits do not increase the dilatation of the heart nor increase its output more than temporarily may also be deduced from the experiments of Hess (5). This author by successive injections of blood maintained an increased blood volume in rabbits for periods as long as $2\frac{1}{2}$ months. At the end of that time the animals were carefully autopsied, but evidence of cardiac hypertrophy was entirely lacking. Hess concluded that there could have been no increase in minute output by the heart and that compensation for the increased volume had been brought about by lenghtening the circulation time.

Hemorrhage after transfusion. Under ordinary conditions the vascular system holds fluid in reserve sufficient to maintain the diastolic filling of the heart until the hemorrhages amount to about 2 per cent of the body weight. It is of interest and also of some practical importance to know whether the same rule holds in an animal that has been rendered plethoric by intravenous injections. The doctrine of plethora does not now have the importance in medicine that it did in the past when almost every condition was believed to be improved by venesection. On the hypothesis of plethora, however, bleeding is still practiced for a dilated right heart accompanied with high venous pressure, although the work of Keith, Rowntree and Geraghty (6) indicates that the total blood volume is probably not increased.

In table 2 may be seen the results of arterial hemorrhage after previous injections. It will be noted that just as in a series without injections, hemorrhages of from 1 to 2 per cent of the body weight have brought about a definite reduction in the cardiac area as determined by the x-ray. As a matter of fact, the actual average percentage necessary for this result is somewhat lower than in our normal series. The area

is in every case below the normal before injection. This may be seen to be true for experiments H 81 and H 85 by referring to table 1. A plethoric condition then does not render the animal any less subject to hemorrhage, as determined by the relative cardiac areas.

It would seem that if the capillaries and venules act as reservoirs after an injection, they would protect the effective circulation and cardiac size from correspondingly larger hemorrhages. For some reason, however, our results do not indicate that such is the case. It may be that the large injections employed injured the walls of the venules and capillaries by reducing their tonus or elasticity.

The relation of venous pressure to cardiac size and output. It must be clearly noted that the results previously described as resulting from

TABLE 2

Data showing the effect of hemorrhages after plethora induced by previous injections of 6 per cent acacia in physiological saline

NUMBER OF EXPERIMENT	WEIGHT	TOTAL INJECTION	HEART AREA AFTER INJECTION	BLEEDING	HEART AREA AFTER BLEEDING
	kilos	*cc.*		*cc.*	
H 81	8.55	350	52.9	100	45.0
H 82	7.00	300	55.6	100	50.7
H 83	6.80	500	39.7	50	33.4
H 84	12.00	1200	71.4	100	56.2
H 85	8.20	800	43.6	100	39.2
H 90	8.20	160	48.4	100	42.3
H 95	11.50	950	66.8	200	54.6
H 96	6.90	650	36.9	200	28.6

intravenous injections and arterial hemorrhages are derived from observations made after the immediate effects of the procedure have passed off. For example a large rapid intravenous injection at once raises venous pressure and often increases the heart rate. After a few minutes, however, the former pressure and rate usually return. It was at this stage that the previous observations were made. We wish now to report on x-ray observations made immediately following the injections, particularly those at various levels of venous pressure.

All students of cardio-dynamics know the large number of investigations which have been made on the factors influencing cardiac output, particularly those of Henderson and his co-workers in which the cardiometer had been employed, and those of the English school in which Starling's heart-lung preparation has been generally used. A detailed review of this literature does not seem called for here since

excellent résumés may be found in Bainbridge (7) and in a recent paper of Wiggers' (8). Suffice it to say that Starling and his collaborators have insisted that cardiac output could be increased independently of rate by the increase in systolic stroke due to increasing the venous pressure, while Henderson and his school have, on the contrary, maintained that under all normal or increased venous pressures the volume curves were superimposable and output was therefore determined, not by increased filling, but by the duration of the cardiac diastole, that is, the heart rate. Above a venous pressure of 50 mm. of water which Henderson regarded as "critical," the right ventricle filled as quickly as it relaxed and the discharge was therefore always maximal for a given rate of beat.

On Henderson's hypothesis that the amplitude of stroke decreases as the rate of beat increases the utmost acceleration of which the circulation is capable would be less than double the resting value (9). Such a conclusion is however out of harmony with the great increases in minute volume which have been found necessary in exercise by Zuntz, Plesch and Krogh. To reconcile these conflicting points of view Krogh (10) has classified the blood supply to the ventricles as adequate or inadequate. An adequate supply is one of sufficient volume and pressure to make the ventricular filling curve show an abrupt rise and a more or less pronounced plateau without any indication of the auricular contraction. This is a condition realized in Henderson's curves when his venous pressure was above the critical point of 50 mm. The chief factor governing the output of such a heart would obviously be the frequency of beat. Krogh believed all of Henderson's experiments were with adequately filled hearts. An inadequate supply is one of insufficient volume and pressure to fill the ventricles at once. The filling curve is therefore less steep and its height is not so great, and the beat of the auricle adds a shoulder to the curve. Under such conditions the output is governed almost entirely by the venous pressure. Hearts, normally, according to Krogh, are inadequately supplied with blood. The increasing venous return in exercise would then first increase the systolic stroke of the heart until the supply became adequate. After that, increased output would be secured by increased rate, according to Henderson's hypothesis.

From his cardiometric and pressure determinations, Wiggers has also criticized Henderson's "Law of uniformity of cardiac behavior." As early as 1914 (11) Wiggers pointed out that venous pressures of 40 to 75 mm. of H_2O were only relatively critical and that the ventricular

volume curves were not entirely superimposable until pressures of 150 mm. were reached. In a recent paper Wiggers and Katz (12) have presented cardiometer curves showing that systolic discharge increases progressively with the rising venous pressure until the latter reaches a range of 250 to 310 mm. Above this there was decompensation. The same authors have also noted that on increasing venous pressure, ventricular systole is prolonged independent of the cycle length. Other references might be given on the general problem but these are sufficient to follow the thread of the argument up to the present time.

In the experiments now to be described variations in venous pressure were secured by injections of 6 per cent acacia in physiological saline into the left external jugular. The pressures themselves were read from a sound inserted into the right external jugular and extending into the vena cava almost or quite to the opening into the right auricle. The effective venous pressure was, of course, always somewhat higher than the figures given.

The height to which venous pressure rises and the length of time it remains above normal depend particularly on three factors; the amount injected, the duration of injection, and the amounts previously injected. The effect of successive injections of equal volumes at a constant rate may be seen in certain of the experiments analyzed in table 3. In experiment H 124 venous pressure as a result of the first injection of 200 cc. rose to 8 cm. and returned to normal in 7 minutes. The second injection raised the pressure to an equal height but the return to normal was delayed for 25 minutes. A third injection raised venous pressure to 10 cm. with a delay in return of 31 minutes. The injections had gradually filled the circulatory reservoirs and with the last injection the venous pressure remained high until the fluid could be disposed of by transudation or otherwise.

The rate of beat has, of course, an influence on the size of the heart, so it was necessary to be sure of the pulse rate at the moment the x-ray pictures were taken. This was secured either from arterial blood pressure records or by means of the electrocardiagraph. An increase in venous pressure is often accompanied by an increase in heart rate. In our experiments this acceleration has been inconstant and seldom large. Its mechanism has been studied by Bainbridge (12) who determined that it was a reflex through the cardiac nerves, chiefly the vagus. Although it is a matter of common experience that slow rates increase the heart size and fast rates decrease it, the exact relation

TABLE 3

Data showing the effect of increased venous pressure on the diastolic size of the heart

EXPERIMENT NUMBER	WEIGHT	TIME	INJECTION	VENOUS PRESSURE	HEART RATE	HEART AREA
	kilos		*cc.*	*cm H_2O*		
		3.39		0.50	210	51.6
		3.46	250	10.00	189	59.5
		4.02		0.25	130	50.7
		4.20	250	10.00		
		4.23		6.50	147	59.6
H 92	10.45	4.34		−1.00	165	51.9
		4.41		−2.50		
		4.41	250	7.75		
		4.42		5.50	162	59.0
		4.51		−2.00	171	51.0
		3.45		1.50	178	54.1
H 93	9.30	3.54	250			
		3.55		1.50	210	53.6
		10.09		1.00	132	32.2
		10.28	200			
		10.29		8.00	159	38.9
		10.33		−1.00	138	30.9
H 96	6.90	10.42	200			
		10.44		6.00	168	38.1
		11.11	250	2.00		
		11.13		19.00	192	39.1
		9.19		1.50	102	31.0
		9.29	100			
		9.31		16.00	90	34.4
		9.48		2.75	90	32.0
		9.57	100			
		9.59		8.00	115	33.7
		10.29		2.75	102	30.9
H 101	6.10	10.30	100			
		10.33		11.00	110	34.9
		11.19		1.50	108	31.7
		11.22	100			
		11.24		9.50	120	33.6
		11.27		5.00	120	32.6
		11.37		1.50	120	31.7
		9.58		1.00	133	47.3
H 124	10.7	10.12	200			
		10.13		8.00	138	52.3

TABLE 3—*Concluded*

EXPERIMENT NUMBER	WEIGHT	TIME	INJECTION	VENOUS PRESSURE	HEART RATE	HEART AREA
	kilos		*cc.*	*cm. H_2O*		
		10.16		4.00	132	48.7
		10.18		2.00	129	48.0
		10.19		1.00		
		10.42	200			
		10.43		7.50	129	52.8
H 124	10.7	10.46		4.75	135	50.7
		10.56		2.00	147	46.2
		11.08		1.00		
		11.18	200	10.00		
		11.20		9.50	162	50.0
		11.33		5.50	159	47.9
		11.44		2.00	171	46.1
		11.49		1.00	171	45.3
		2.59		−2.00	174	46.7
		3.20	300			
		3.22		9.00	162	63.6
H 125	13.30	3.22½		7.00	162	60.0
		3.25		4.00	140	61.5
		3.31		0.50	138	51.7
		3.56		−1.75	180	46.8
		3.44		−1.00	120	30.2
		4.05	350			
		4.00		5.00	120	31.2
H 126	5.12			13.00	156	31.7
				21.00	166	32.8
		4.07		37.00	168	32.9
		4.10		4.00	168	30.7
		2.39		−1.00	219	28.2
		2.49	350			
		2.50		7.00	170	35.7
H 127	5.56			13.00	204	39.5
		2.51		25.00	214	37.6
		2.51½		30.00	210	39.4
		2.53		7.00	168	36.4

between heart rate and heart size in the intact animal, other conditions being kept constant, does not seem to have been adequately investigated. Our experiments seem to indicate that the differences in rate must be rather large before an effect is noted on heart size.

In two preliminary experiments we determined the cardiac output during periods of increasing venous pressure by means of a cardiometer which was connected to a large capacity tambour. The results showed a steady increase in diastolic size and cardiac output as pressures increased from 3 to 130 cm. of H_2O. The results were identical with those just obtained by Wiggers with more accurate methods and need for that reason detain us no longer here.

The use of the x-ray in studying the effect of increased venous pressure on cardiac size and output has the very great advantage of leaving the thorax of the animal intact. The heart-lung preparations and the cardiometric experiments show unquestionably what the heart can do but just how far the conclusions can be applied to that organ while in the enclosing chest and the restraining pericardium has always been somewhat of an open question. X-ray photographs, taken with the tube at least a meter's distance from the plate, give a silhouette area of the dog's heart which is quite free from distortion. The dog is a particularly favorable subject since the apex of the heart is usually almost and often quite clear of the diaphragm. Pictures may be taken successively with variations in the silhouette area under 5 per cent, although we have usually allowed that much before attaching significance to it. The areas are measured by the planimeter and expressed in square centimeters. Since hearts are quite uniform in shape the silhouette area must bear some relationship to the mass of the heart itself. This relationship Bardeen (14) has worked out for the human heart and Skavlem (2) has now done the same for the dog's heart. A change then in the heart size may be noted by comparing the silhouette area with the normal or by reducing these to their appropriate volumes. Such a method of determining cardiac size does not have the fine degree of accuracy that is given by cardiometers properly used nor their continuity of record, but it is quite accurate enough to show in which direction the result lies and it has the tremendous advantage of leaving the heart undisturbed in all its relations. Henderson (9) himself once proposed to attack the problem by this method.

In table 3 may be seen the results of x-ray determinations on hearts at varying degrees of venous pressure. The areas represent the diastolic sizes and were secured by three short flashes taken during the inspiratory phase. It will be noted at once that all increases in venous pressure are associated with increases in the diastolic heart area over the normal. In certain exceptional cases such as experiment H 127, the diastolic heart area may be enlarged in this way nearly 40 per cent.

The dilatation is not a result of changes in rate for in experiment H 124 there was a 10 per cent variation in size with a change of only 5 beats per minute in rate while in experiment H 96 the heart increased in size in spite of its acceleration.

In a general way, our series show that the diastolic size of the heart increases correspondingly with the venous pressure up to 100 mm. H_2O and very slightly or not at all beyond this point. In experiment H 101, for example, the heart sizes at pressures of 11 and 16 are not materially different. The same may be said of pressures 8 and 19 in experiment H 96 and of pressures 7 and 30 in experiment H 127. In experiment H 126, on the contrary, there is a definite increase in heart size as venous pressure rose from 13 to 21. On the whole, our results on this point agree with those of Patterson, Piper and Starling and Wiggers, and are opposed to the interpretations of Henderson and his collaborators. Diastolic volume increases with increasing venous pressure to a point far beyond the "critical pressure" of 50 mm. If the negative intrathoracic pressure be estimated at 50 mm., the average venous pressure beyond which we could find no increase in diastolic size would be about 140 or 150 mm. H_2O. This level is considerably lower than that set by Wiggers, who found that ventricular discharge and diastolic size increased to somewhere between 250 and 300 mm. of saline. This difference does not seem attributable to our method which might quite as easily show further changes in heart size. It is possible that it depends on the intact pericardium. After a certain point it seems from the work of Evans and Matsuska (15) that the pericardium acts as a restraint on further dilatation. In cardiometer experiments with the chest open one might well find dilatation above the level which would be critical for the intact animal.

Although the data presented in table 3 clearly show that the filling curves of the ventricles are not superimposable at least until a very considerable venous pressure is reached, they do not give us any real evidence concerning output. The inference would be that output per beat increased with the greater diastolic size but this advantage might of course be overcome by a reduced systolic stroke. As a matter of fact the cardiometer volume curves do show an increased systolic size with rising venous pressures, although the systolic size rises slower than the diastolic, and the output per beat is therefore increased.

To obtain a measurement of output with the x-ray, we have used the method already described by us (16) for obtaining instantaneous pictures of the heart. By means of a rapid plate changing device and

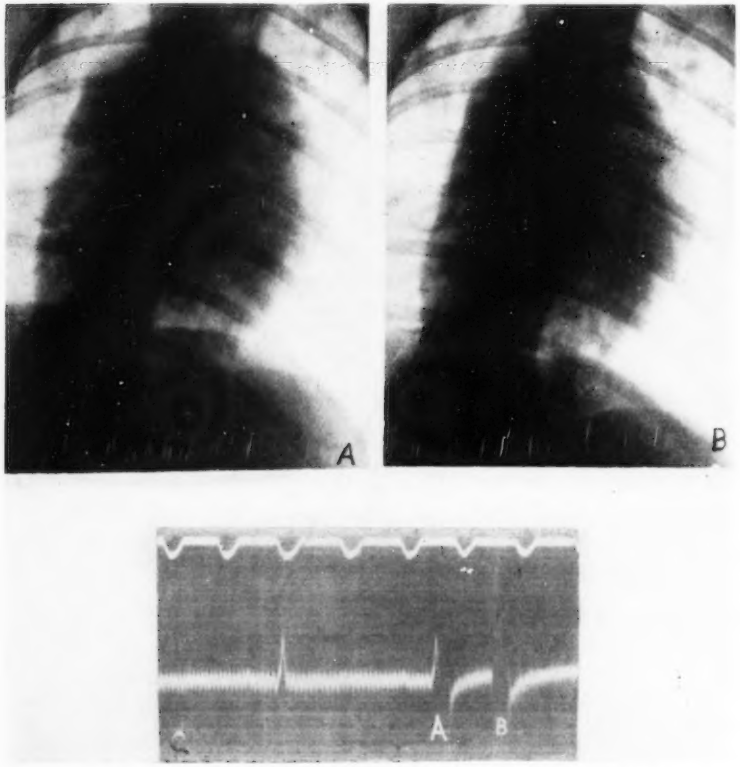

Fig. 2. To illustrate the method of determining cardiac output by means of the x-ray. Exposure A as shown by a simultaneous electrocardiagram was taken during the downstroke of the R wave and exposure B was made in the same cycle at the end of the T wave. These points mark the maximum diastolic and systolic sizes of the heart. The first exposure lasted about $\frac{1}{10}$ second, the second was a trifle longer. The T wave in the electrocardiagram submitted is not clear due to the tight string necessary to receive the induction shocks from the x-ray discharge. The exact location of this wave is known, however, from records made immediately afterwards with a loose string. The difference in size between the two exposures is obvious to the eye. These exposures gave the data for the normal output in experiment H 113.

circuit breakers, we have, in 5 experiments, secured two pictures in the same cardiac cycle, one set before and the other after a rise of venous pressure. The first of each pair of these exposures, as shown by simultaneous electrocardiograms, was taken during the R wave and the second at the end of the T wave. They therefore give cardiac silhouettes during the periods of rising and falling tension, at which times only may one secure the true diastolic and systolic outline of the heart.

TABLE 4

Data showing effect of an increased venous pressure on systolic and diastolic size and cardiac output per beat. The areas were obtained from x-rays taken during the periods of rising and falling tension with exposures of about $\frac{1}{10}$ second

EXPERIMENT NUMBER	WEIGHT	INJECTION	HEART RATE	VENOUS PRESSURE IN	DIASTOLIC AREA	DIASTOLIC VOLUME	SYSTOLIC AREA	SYSTOLIC VOLUME	OUTPUT PER BEAT
	k. lbs.	*cc.*		*cm. H_2O*	*sq. cm.*				
H 112	11.00	300	150	1.00	44.6	131.0	41.7	118.4	12.6
			160	4.00	55.0	179.4	51.3	161.6	17.8
H 113	10.00	300	126	1.00	49.4	152.7	47.4	143.7	9.0
			114	17.00	52.7	168.4	49.5	153.5	14.9
H 114	10.20	200	117	1.00	40.6	113.9	37.6	101.6	12.3
			120	8.00	42.3	121.0	38.1	103.5	17.5
H 115	10.80	250	168	−2.00	49.2	151.8	46.8	140.8	11.0
			180	6.50	57.8	193.5	54.1	175.2	18.3
H 116	11.80	200	135	0.50	40.7	114.2	38.5	105.1	9.1
			130	6.50	40.9	155.1	44.8	131.9	23.2

From these areas the volumes during diastole and systole have been computed, the difference giving the output per beat. In table 4 may be seen these determinations. It will be readily seen that the increased venous pressures have resulted in each experiment in an increased output per beat. The results justify the conclusion that an increased diastolic area due to rising venous pressure is indicative of an increased output. The data are not abundant enough to show at what level, if any, output ceases to increase with rising venous pressure. The technical difficulties prevent x-rays being taken frequently enough to settle this point. That the critical level, if it exists, is well above that of 50 mm., however, seems evident. Three of the experiments had venous pressures of 10 mm. for their normal. If to this we add

the negative intrathoracic pressure, we have in these cases an effective pressure of some 50 to 60 mm., yet these hearts, on a further raise of venous pressure, at once responded by increased output. Our work indicates that in order to increase its output the heart may react to a rising venous pressure in two ways, by an acceleration in rate, or by an increase in diastolic size and output per beat. As a matter of fact, our records show that usually both of these mechanisms are employed at the same time. In experiment H 93, table 3, there is an example, however, of an injection of 250 cc. which did not raise venous pressure apparently because of the accelerated heart rate. The diastolic size of the heart also remained unchanged which showed that in this case output was taken care of by an increase in rate alone.

SUMMARY

Intravenous injection of acacia saline, physiological saline, or blood, in quantities varying from 25 to 103 per cent of the total blood volume was not found to increase permanently the diastolic size of the dog's heart. Hemoglobin determinations indicated that much of the fluid was still in the circulatory system. Photomicrographs of the capillaries and venules of the ear following injection showed that these vessels were acting as reservoirs and thus preventing a cardiac dilatation or an increase in the volume of the effective circulation.

Animals rendered plethoric by intravenous injections were found as susceptible to hemorrhage as normally. That is, in this condition bleedings of 1 to 2 per cent of the body weight still continued to reduce the cardiac diastolic size.

The immediate effect of an intravenous injection was a rise in venous pressure and this was always associated with an increase in the diastolic size of the heart. The heart continued to dilate with the rising pressure until the latter reached something like 150 mm. H_2O. Beyond this pressure we found little effect on diastolic size.

During the period in which an intravenous injection raised venous pressure and increased the diastolic size of the heart, we have found an increased output per beat as determined by the x-ray method.

These results indicate that the filling of the ventricles may vary with the venous pressure up to 150 mm. H_2O independently of the rate. With a rising venous pressure and an augmented inflow the cardiac minute volume may be increased by a longer systolic stroke as well as by a more rapid rate.

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